Steven M. Ruben
Appl. No. 10/662,429

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Department Mol. Bio.
Subject 10/20/94 – 02/02/95.
Name ANN KIM #8.
Address

National Brand 43-648

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701

75 Sheets
11¾" x 9¼"
4x4 Quad.

0 73333 43648 8

Ruben EXHIBIT #91

HTPB411 & HTPAN308 in PD10

Pg150 Book 7

10/20/94

Ligations

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
HTPAN308 9/30	4	4	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10/20	—	—	—	6	6	6	—	—	—	—	—	—	—	—	—	—	—	—	—
HTPB411 9/30	—	—	—	—	—	—	9	9	9	—	—	—	—	—	—	—	—	—	—
10/20	—	—	—	—	—	—	—	—	—	6	6	6	—	—	—	—	—	—	—
PD10 1:1	1	—	—	—	—	—	—	—	—	—	—	—	6	6	6	—	—	—	—
PD1024 10/17	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—	—
10/19	—	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—
10/19	—	—	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—	2	—
10X Buffer	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
H_2O	11	11	11	9	9	9	12	12	12	9	9	9	9	9	9	15	15	15	17
T4 Ligase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

Incubate @ 16°C O/N

10/21/94

Transform into M15 Chemically Competent Cells

Thaw M15 Cells on ice
Aliquot 100µl into 6 fresh Sterile tubes
Add 10µl of Ligation Rx Tubes.
Incubate on ice 1 hr
Heat 42°C 45 sec
Place on ice
Add 400µl LB
Incubate 37°C 1 hr
Plate 175µl onto LB+ Amp + Kan plates
Incubate RT over the weekend

Department Mol. Bio.

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0 73333 43648 8

75 Sheets
11¾" x 9¼"
4x4 Quad.

Ruben EXHIBIT 2091
Ruben v. Wiley et al.
Interference No. 105,077
RX 2091

4 HTPB411 & HTPAN08 in PDI0

4/94

Plates look OK.

pPI0 1.1 + 2.4 looks very good so ligations worked well

pPI0 Vector colonies had many Colonies
So Did Vector + PDH6 fragment
Try PCR on colonies.

Pick 200 each goto 200 ul LB+Amp+Kan in 96 well dish - HTPAN08 + HTPB411

Incubate 37°C 4hrs w/aeration

PCR.

HTPAN08

		200x
10X PCR	3.2	640
10XdNTP	3.2	640
2499	0.2	40
2500	0.2	40
Taq	0.2	40
H_2O	23	4600
Cult.	2	

HTPB411

		200x
10X PCR	3.2	640
10xdNTP	3.2	640
2501	0.2	40
2502	0.2	40
Taq	0.2	40
H_2O	23	4600
Culture	2	

PCR Program #609.

95°C 5min
95°C 20sec
55°C 20sec } 30x
72°C 1min
72°C 7 1/2 min.
4°C Hold.

10/24/94

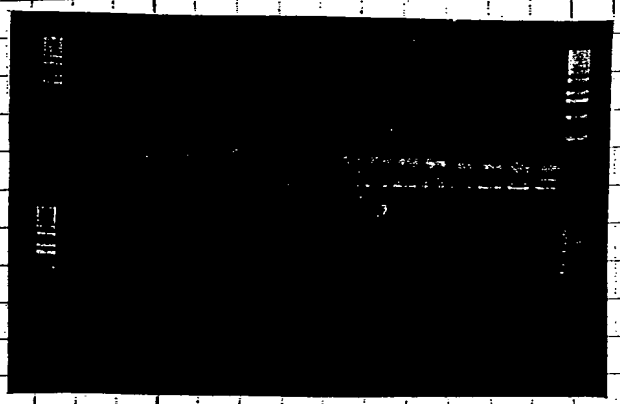
Run 10 µl on gel w/ 1 Kb ladder

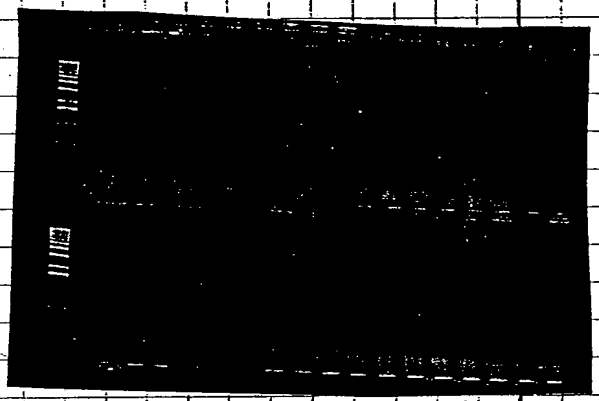
– Nothing Worked!

Didn't Bother to take pictures.

HTPAV08

had strong size band.





Retry – Make New Primers today later.

– Phenol extract from PCR – then Ethanol ppt – Digest O/N.

– Run on LMP gel.

10/26/94

Try using 5' Bam primers from ligation into pAZ Vector.

HTPAV08 – 7689

HTPB411 – 2429.

6 HTPAN08 + HTPB4[1] in PDI

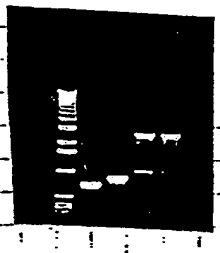
10/26/04 DNA long gel.

	HTPAN08 ①	HTPAN08 ②		HTPB4[1] ③	HTPB4[1] ④
10xPCR	10.0	10.0	10xPCR	10	10
10xdNTP	10.0	10.0	10xdNTP	10	10
2499	10.5	0.5	2501	0.5	0.5
2500	0.5	—	2507	0.5	—
7689	—	0.5	2429	—	0.5
Taq	0.3	0.3	Taq	0.3	0.3
H_2O	77.7	77.7	H_2O	77.7	77.7
DNA	1	1	DNA	1	1
	100	100		100 µl	100 µl

Run PCR Program #04. Modified

95°C 5 min
95°C 20 sec
55°C 20 sec
72°C 1 min
] 25X
72°C 7 1/2 min.
4°C Hold

Run 5 µl on gel with 1Kb ladder



looks good.

Add equal Volume PEG/NaCl
Slt on ice 10 min
Spin 10 min
Remove Supernatant
1X 70% Ethanol wash. 500 µl
Spin 5 min
Remove Supernatant.
Let pellet Dry Slightly
Resuspend in 20 µl TE
Add equal Volume Phenol
Vortex

HTPAD08 + HTPB[illegible] in RNO 7

10/26/94

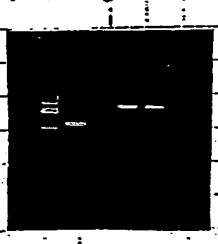
Spin 5 min
Transfer upper Aqueous layer to fresh tube.
repeat.
Add 2 volumes Ethanol (400 µl)
1/10 volume 3M NaOAc. (20 µl)
Mix
Let sit on ice 1/2 hr.
Spin 10 min
Remove supernatant.
1 X 70% Ethanol wash ~1000 µl
Spin 5 min.
Remove supernatant
Allow pellet to dry
Resuspend in 200 µl TE.
Set up digestions.

DNA	100 µl.
10X #2	20 µl
H_2O	79 µl
Xba/Bam	0.5/0.5
	200 µl.

Incubate 37°C / overnight

10/27/94

Run 10 µl on 1% gel with 1 Kb ladder.

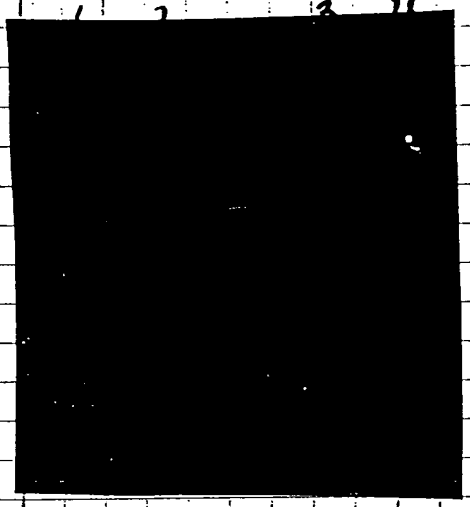


Gel looks good
Add Sample Buffer and
Run on 0.8% LMP
Agarose gel
Run 80V 1/2 hrs.

Ethanol ppt Samples (see above) - 1 X 70% Wash
Resuspend in 40 µl TE - Now Ready for gel

8 HTPAV08 & HTPB411 in PD10

10/27/94

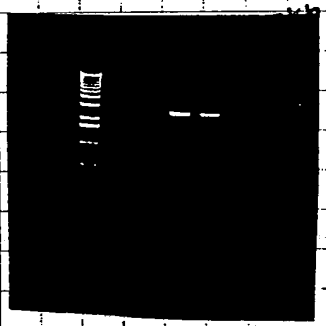


for Sample #1 cut out 1.8 Kb fragment too

Gene Clean
Add 1000 µl NaI
Heat 55°C 5 min
Mix well
Add 5 µl Glass milk
Let incubate at Room temp 5 min w/ occasional mixing
Spin 7 sec.

3X [Remove Supernatant
Resuspend pellet in 400 µl Wash Buffer
Spin 7 sec
Remove Supernatant]
Spin 7 sec
Remove Supernatant
Resuspend pellet in 20 µl TE
Heat 55°C 1 min
Spin 7 sec
Transfer to fresh tube
Repeat 20 µl TE

Run 2 µl on gel with 1 Kb Ladder



Use to Set up ligations

DO Not use the 1.3 Kb fragment from #1.

10/27/94

Ligation Reactions.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
HTPA108 #1	4	4	—						4							
HTPA108 #2	—	—	4	4	—					4						
HTPB411 #3	—				4	4				—	4					
HTPB411 #4	—						4	4				4	—			
pD10 1.1	—													—	—	4
pD10 10/19	1	—	1	—	1	—	1	—	—				1	—	—	16
pD10 10/12	—	1	—	1	—	1	—	1	—					1	—	—
10X Buffer	2	2	2	2	2	2	2	2	2	2	2	—	—	—	—	—
H2O	12	12	12	12	12	12	12	12	13	13	13	13	16	16	17	12
T4 Ligase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

Incubate at 16°C O/N.

10/28/94

Transform M15 Chemically Competent cells.

Thaw cells on ice
Aliquot 100µl of cells into fresh tubes.
#16 only got 250µl.
Add 10µl of ligation to tubes
Incubate on ice 1 hr.
Heat 42°C 45 sec
Put on ice
Add 400µl LB
Incubate 37°C 1 hr
Plate onto LB + Amp + Kan plates. 150mm.
plate 1-16 100µl.
1-8 250µl also
plate #16 (100µl) mixed w/ #7.

10 HTPAN08 & HTPBY11 in PDID

10/28/94

Incubate 37°C O/N.
Asked Paul Moore to place at 4°C
Tomorrow.

10/31/94

Paul did not take plates out
- there were colonies. So try to PCR
Pick 2400 each 1-8 & PCR

	1+4 HTPAN08				5-8 HTPBY			
	1-2	50	3-4	50	5-6		7-8	
10X PCR	3.2	160	3.2	160	3.2	160	3.2	160
10 xdNTP	3.2	160	3.2	160	3.2	160	3.2	160
2499	0.2	10	0.2	10	2501 0.2	10	2501 0.2	10
2500	0.2	10	0.2	10	2502 0.2	10	2499 0.2	10
Taq	0.2	10	0.2	10	0.2	10	0.2	10
H2O	23	1150	23	1150	23	1150	23	1150
DNA	8							
Cult	2	—	2	—	2		2	

PCR Prog 69.
Pithing set up.
Remake.
Try by pow.

PD 13

HTPB411 & HTPAN08 in PD10

Pg 12

10/31/94

Make more insert again
PCR w/ New and old primers.

	(1)	(2)	(3)	(4)
HTPAN08				
3'Xba 2499	0.2	—	0.2	—
3'Xba 2656	—	0.2	—	0.2
5'Bam 2500	0.2	—	—	0.2
5'Bam 7689	—	0.2	0.2	—
10X PCR	10	10	10	10
10X dNTP	10	10	10	10
Taq	0.2	0.2	0.2	0.2
DNA (10ng/μl)	1	1	1	1
H_2O	78.4	78.4	78.4	78.4
	100	100	100	100

	(5)	(6)	(7)	(8)
HTPB411				
3'Xba 2501	0.2	—	0.2	—
3'Xba 2653	—	0.2	—	0.2
5'Bam 2502	0.2	—	—	0.2
5'Bam 2429	—	0.2	0.2	—
10X PCR	10	10	10	10
10X dNTP	10	10	10	10
Taq	0.2	0.2	0.2	0.2
DNA (10ng/μl)	1	1	1	1
H_2O	78.4	78.4	78.4	78.4
	100	100	100	100

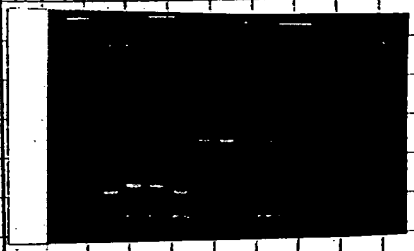
PCR Prog #619

95°C	5min	
95°C	20 sec	25X
55°C	20 sec	
72°C	1 min	
72°C	7 1/2 min	
4°C	Hold	

14 HTPA008 & HTPB411 in PD10

1/1/94

Ran 5ul on gel with 1Kb ladder.



Add equal Volume PEG/NaCl
Spin 15 min
70% Ethanol Wash.
Resuspend 100ul.
Digest O/N.

DNA	50ul
H_2O	38
10x #2	10
Xba/Bam	1/1
	100ul

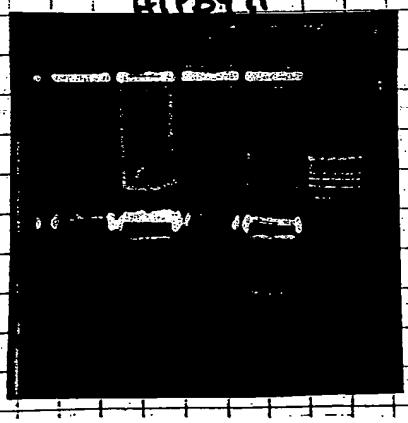
Incubate reaction 3
37°C Overnight

11/2/94

Run Precipitate Digest
Add 1/10 Vol 3M NaAcetate
2 Vol 100% Ethanol
Spin 15 min
70% Ethanol Wash.
Allow pellet to dry
Resuspend in 45ul of TE
Add 5ul 10x Sample Buffer.
Run on 0.8% L.M.P. Gel.

HTPA008

HTPB411

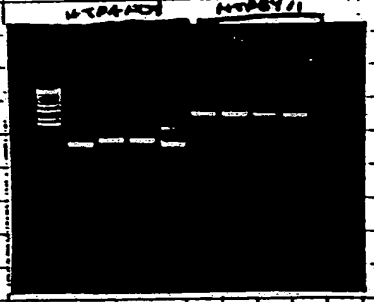


HTRA2OS + HTPB411 in pSIO

1/2/94

Cor
Cut out fragment
Gene Clean
Add 1000 ul pdI
Heat 65°C 2 min
Mix well
Add 7 ul Glass milk
Incubate at Room Temp 5 min
With occasional Mixing
Spin 7 sec
Remove Supernatant
3X [Resuspend pellet in 400 ul Wash Buffer
Spin 7 sec
Remove Supernatant]
Spin 7 sec
Remove Supernatant
Add Resuspend pellet in 20 ul TE
Heat 65°C 1 min
Spin 10 sec
Transfer to fresh tube
Repeat with 20 ul TE

Run 2 ul of eluted fragment on 1% TAE gel with 1 Kb ladder



With fragments from 10/27
And the new fragments
Set up ligations

16 HTRANS08 HTRP5H11 into pD10

11/2/94

ligation Reactions

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HTRANS08 2499+2500 6/22	3														
2499+7689		3													
11/2 2499+2500			3												
2656+7689				3											
2499+7689					3										
2656+2500						3									
HTRP5H11 2501+2502 6/27							3								
2501+2429								3							
11/2 2502+2502									3						
2653+2429										3	3				
2501+2429												3			
2653+2502															3
pD10 BamHI	1	1	1	1	1	1	1	1	1	1	1	1	1	—	1
10X Lig Buffer	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
H_2O	13	13	13	13	13	13	13	13	13	13	13	13	14	17	13
T4 Ligase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
pD10 9.1kb															3

Incubate 16°C O/N.

11/3/94

Transform M15 cells
use 1-8 from 10/27 ligations
as well as 1-15 from 11/2

To 100 µl of cells add 10 µl of ligation
Let sit on ice 1 hr
Heat 42°C for 45 sec
Add 1000 µl LB
Incubate 1 hr at 37°C
Plate onto 2 LB + Amp + Kan plates

HTPAN08 + HTPB411 in pD10

11/3/94

150 mm plates -
use 150 ul 1-8 & 1-12
use 300 ul 1-8 & 1-12

Incubate 37°C O/N.

11/4/94

Pick 1-8 10/27 [illegible]
into 200 ul LB + Kan + Amp plates
pick 1-12 11/2 into
[illegible] 200 ul LB + Amp + Kan

Incubate 37°C w/ aeration
Do PCR.
use internal primers to confirm

HTPB411 RP10 + FP15
HTPAN08 RP12 + FP18.

HTPB411

		200X
2400	0.25	50
7945	1	200
10X PCR	3.2	640
10X dNTP	3.2	640
Taq	0.2	40
H_2O	22.15	4430
Culture	2	

HTPAN08

		200X
6638	0.25	50
7407	0.25	50
10X PCR	3.2	640
10X dNTP	3.2	640
Taq	23.1	4620
H_2O	0.2	40
Culture	2	

PCR Program H69

95°C 5 min
95°C 20 sec ⎤
55°C 20 sec ⎥ 25X
72°C 1 min ⎦
72°C 7½ min
4°C hold

Store [illegible] at -20°C [illegible]

(+) Control Plasmid
(-) Control H_2O

15
1
2
13
1
3

18 HTPAN08 + HTPB411 in PDTO

11/9/94

Ran 10 µl of Reaction on gel with (1kb ladder)

Nothing Het up again!

Talked w/ Steve -
Need to remake primers,
Sites on old ones wrong.
Submitted both HTPAN08 + HTPB41
5' Bam & 3' Xba.

11/8/94

3' Xba HTPB411 - Oligo Rec'd.
(60°C O/N).

11/10/94

Rec'd 5' Bam New HTPB411,
5' Bam New HTPAN08.

11/11/94

Rec'd 3' Xba New HTPAN08.

11/12/94

Dried Oligos
Ran PCR Reactions w/ new Primers

(P.29)

HTPAN08 / HTPB411 in PD10

pg 18

11/14/94

HTPAN08S04		HTPB411S15	
DNA (10ng/µl)	1	DNA (10ng/µl)	1
3'Xba Nest	0.6	3'Xba Nest	0.6
5'Bam Nest	6	5'Bam Nest	6.7
10X dNTP	10	10x dNTP	10
10X PCR	10	10X PCR	10
H_2O	72.1	H_2O	71.4
Taq	0.3	Taq	0.3
	100		100

PCR Program #69 modified

95°C	5min	
95°C	20sec	25X
55°C	20sec	
72°C	1min	
72°C	7½ min	
4°C	Hold	

H/H Run 5µl on gel w/ HB Ladder

↑ 2nd Digest

Add equal Volume PEG/NaCl
Spin 10 min
Pour off Supernatant
Wash 60 1ml 70% Ethanol
Spin 5 min
Rinse Supernatant
Resuspend in 100µl TE
Digestions

DNA	25
H_2O	19
10X #2	5
Xba/Bam	0.5/0.5
	50µl

Incubate RT ~~o/n~~ till Monday 11/14

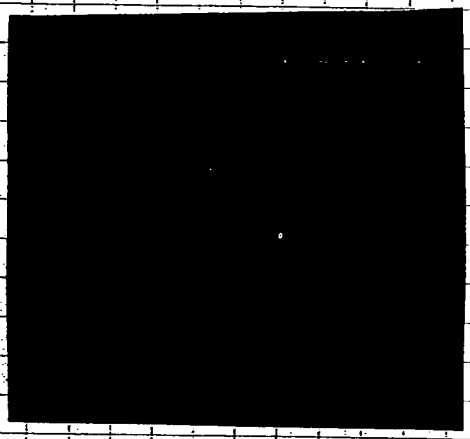
40 HTPAV08 | HTPB411 in PDK0

11/14/94

Restrict

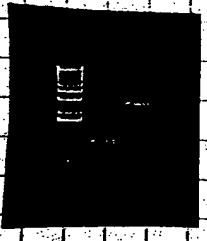
Run digest on gel - See pg 39.
with 1kb ladder

Run Digest on 0.8% LMP Gel
Run 80V 2hrs



Cut out fragments
take picture
Use Qiaquick Gel Extraction Kit
- Add 900 ul QX1 Buffer
- Incubate 60°C 10 min
- Place in Spin Column
- Spin 60 sec. - Remove Flowthrough
- Add remaining Gel/QX1
- Spin 60 sec.
- Remove Flowthrough

Add 750 ul Buffer PE
Spin 60 sec
Remove flowthrough
Add 750 ul Buffer PE
Spin 60 sec
Remove flowthrough
Spin 60 sec
Transfer Column to fresh tube
Add 50 ul TE Heated 60°C
Spin 60 sec
-Run Qiaex gel with 1kb ladder



looks good.

HTPAN08 / HTPB411 in PD10

11/14/94

Set-up ligations

	1	2	3	4	5
HTPAN08	4	—	—	—	—
HTPB411	—	4	—	—	—
PD10 1.1	—	—	4	—	—
PD10 2.4	1	1	1	1	—
10X Buffer	2	2	2	2	2
T4 Ligase	1	1	1	1	1
H_2O	12	12	12	16	17

Set up 2 sets - 1 for 16°C overnight
A - 1 set for RT 1 hr.

For A - 1 hr at RT

10 µl ligation
100 µl M15 Chem Competent Cells

Let sit on ice 1 hr
Heat 42°C 45 sec
Place on ice
Add 400 µl LB
Heat 37°C 1 hr
Plate onto LBT Amp + Kan plates
100 mm - 50 µl
@ 150 mm - 200 µl
Incubate 37°C O/N

11/15/94

Plates look OK
Very few colonies on Vector Alone
No colonies on Lig Rxn alone

42 HTPA8508 / HTPB411 in PD10

11/15/94

PCR Colonies from 1, 2 & 3 into LB + Amp + Kan.
Incubate 37°C 4 hrs.

Run PCR Runs

HTPA08504		HTPB411S#5	
3' Xba New	0.2	3' Xba New	0.2
5' Bam New	2	5' Bam New	2.5
10x dNTP	3.2	10x dNTP	3.2
10x PCR	3.2	10x PCR	3.2
H2O	22	H2O	20.7
Taq	0.2	Taq	0.2
Culture	2	Culture	2
	32		32

(+) Control
PCR'd insert -
(-) Control LB + Amp + Kan
with vector alone Colonies
Use both sets of primers & make sure
NO contamination by fragments

Run PCR Program #69.

95°C	5 min	
95°C	20 sec	30x
55°C	20 sec	30x
72°C	1 min	30x
72°C	7 1/2 min	
4°C	Hold.	

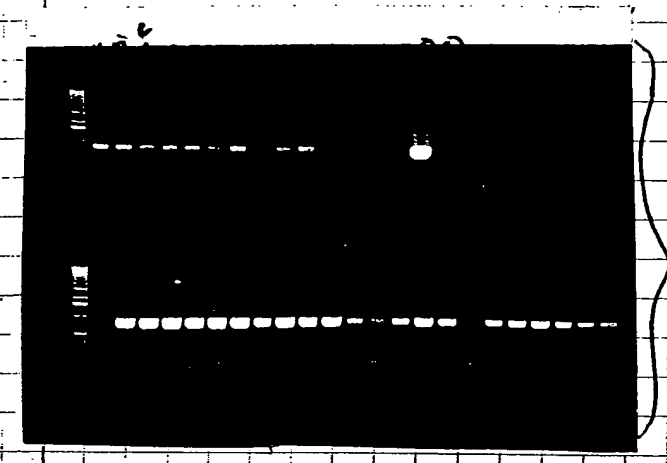
Run 10 ul on gel with 1 Kb ladder
use 1% Agarose gel in TBE

pg 47

HTPAN08/HTPB411 in PD10.

(pg 42)

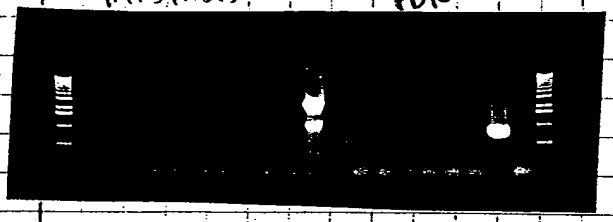
11/15/94



HTPAN08S04

HTPAN08S04
-looks good.
HTPB411S05
Nothing to Re plate / Captains from 16°C O/N.

HTPAN08S04 -
Inoculate 5ml TBt Amp+Kan with 15ml of culture 2-11
Incubate 37°C O/N.



With ligation Rxns left at 16°C O/N

Thaw MS chemically competent cells on ice
To 100 µl of thawed cells add
10 µl of ligation Rxn
to 1 tube #6 add 10ng PD10 ~~plasmid~~ plasmid DNA as ⊕ control
Incubate on ice 1 hr.
Heat 42°C 45sec
Place on ice
Add 400 µl LB
Incubate 37°C 1 hr.
Plate onto LBt Amp + Kan plates
- 100 mm plates - 50 µl
- 150 mm plates - ~~200~~ 250 µl.
Incubate 37°C O/N.

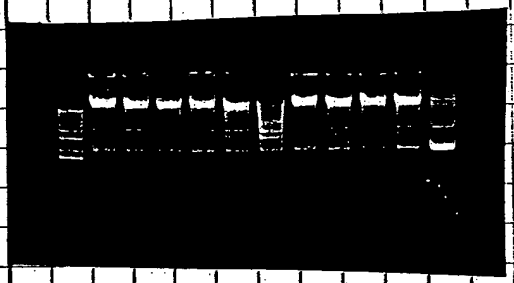
48 HTPB411 & HTPAN08 pD10

11/16/94

Boiling Mini preps

Spin cultures 2min
Remove Supernatant
Resuspend pellet in 750ul STET
+ RNase + Lepozyme
Boil 45sec
Spin 10 min
Remove Pellet
Add equal Volume (750ul) of
13% PEG / 1.6M NaCl
Mix Well
Spin 10min
Remove Supernatant
Add 1000ul 70% Ethanol to clean
pellet
Spin 5min
Remove ~~pellet~~ supernatant
Allow pellet to dry slightly at RT.

Resuspend in 200ul TE
Run 2ul on gel with 1Kb ladder
and pD10



plasmid looks good.
Looks like alot of chromosomal.

Digest with BamH1 to see if insert will "Pop" out of N800 bp

pg 53

HTPAU08 + HTPB4/1 1→ RDO.

pg 48

11/16/94

DNA	5ul
H_2O	17.1
10x #2	2.5
Bam	0.2
Xba	0.2
	25ul

Incubate 37°C 4hrs
Run on gel with 1Kb ladder
Digest pPIO - Should appear @ 1.1Kb fragment

looks like allele
Contact
Submit for Sequencing
with RB10 5'
primer

FASPD01-10 RP01

Set up ligation for HTPB4/1

Fragment	16
Vector	1
T4 ligase	1
10X Buffer	2
	20ul

Incubate RT 5 hrs

Thaw MI5 cells on ice
To 100ul of MI5 chemically Competent cells, Add 10 ul of ligation Reaction + Set up all of ligation.
Incubate on ice 1hr
Heat 42°C 45sec
Place on ice
Add 900 ul LB
Incubate 37°C 1 hr
Plate 250 ul onto LB + Amp + Kan a plates - 150 mm

54 HTPAN08 + ~~HPRGG9~~ / HTPB411 in PDI0

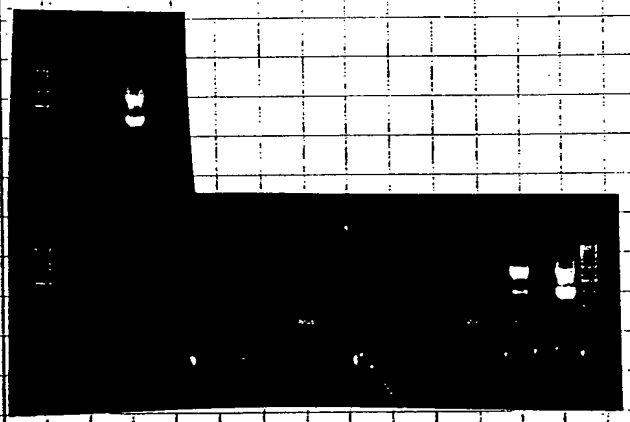
11/17/94

Some Colonies grew.
Pick into 200 µl LB + Amp + Kan
in 96 well dish.
Incubate 37°C 4hrs
PCR -

3'Xba	0.2
5'Bam	2.5
10xPCR	3.2
10xdNTP	3.2
Taq	0.2
H_2O	20.7
Culture	2
	32.

PCR Program #69
use HTPB411515
3'Xba new / 5' ~~Bam~~ New
fragment as
positive Control

Run 10 µl on gel with 1Kb ladder.



looks like
1 positive !!!

Inoculate
50 ml TB + Amp + Kan
with #20
Incubate 37°C
O/N

11/18/94

Qiagen Midi prep -
Spin Culture 4.5K 15min
Pour off supernatant
Resuspend in Tris P1

HTPA008 & HTPB411 in PD10

11/18/94

Add 7ml P2
Mix gently
Add 5ml P3
Mix
Set on ice 20min
Spin 4°C 15min
Equilibrate Tip-100 with 5ml QBT
Apply Supernatant to tube through Resin
Allow to flow through
2X Wash Column 10ml QC Buffer
Elute DNA 10ml QF
Add 7ml Isopropanol (0.7 volumes)
Mix well
Spin 5K 30min
Remove Supernatant
Wash 10ml 70% Ethanol
Spin 5K 15min.
Pour off Supernatant carefully.
Let pellet dry at RT
Resuspend in 200ul TE.
Run 1ul on gel with 1Kb Ladder

Dilute 1:200 in H_2O
Read D_{260}/280

Sample ID	abs 260.0 nm	abs 280.0 nm	blg abs 320.0 nm	260.0 nm 280.0 nm	280.0 nm 260.0 nm
1	0.0557	0.0350	0.0031	1.6927	0.5908

a Stamford

HTPB411 PD10 ~ 160 ug total

Digest with Bam / Vbc to prep with 2.4Kb fragment.

DNA	4	Incubate 37°C
H_2O	13.6	2hrs
10X	2	
Bam	0.2	
Vbc	0.2	

56 HTPAD08 + HTPBY11 in PD10

11/18/94 Run 5ul on gel with 1/2 loading dye & 1ul of undigested.

11/28/94

Made New PAZ Primers 5'end for HTPAD08.

2792 5' Bam 51bp New 7.1pmol/ul
2793 5' Bam 185bp New 8pmol/ul
Use 3' Asp from pervious PAZ cloning 7690

PCR.

5' Bam	3
3' Asp	0.5
10x PCR	10
10x dNTP	10
H_2O	75.2
Taq	0.3
DNA (1ng/ul)	1
	100

[6X]

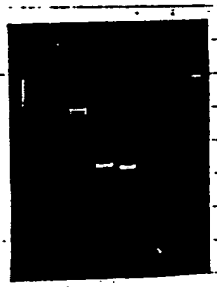
PCR Program #69.

95°C 5 min
95°C 20sec
55°C 20sec
72°C 1min
72°C 7 1/2 min
11°C Hold
] 25X

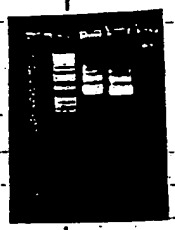
HTPAN08 + HTPBY11

11/21/94

Rem 5ul on gel with 1Kb ladder



Add equal volume PEG/NaCl-mix
Spin 10 min
Remove supernatant
Wash pellet 1ml 70%
Ethanol
Resuspend in 30ul TE
Rem 2ul on gel with 1Kb ladder



Digest fragment
Bam/~~Hba~~

DNA	20
H_2O	23
10X#2	5
Bam	1
~~Asp~~ Xba	1
	50ul

Digest O/N
Store -20°C

11/28/94

Digested w/ Xba by mistake.
Red Digest with

Bam/~~Hba~~ Asp.

DNA	20
H_2O	23
10X#2	5
Bam	1
Asp	1
	50ul

Incubate O/N 37°C.

58 HTRANS6 +PA2 lig

11/30/94

Isolate on 0.8% LMP Gel & Qiaex Clean.
see pg 62

Set up ligations

185 bp Bam/Asp	10	—	—
51 bp Bam/Asp	—	10	—
PA2 Bam/Asp	1	1	1
10x Buffer	2	2	2
T_4 Ligase	1	1	1
H20	16	16	16
	20	20	20

Incubate 16°C O/N.

12/1/94

Transform into M15 cells

Thaw M15 Chem. Competent Cells
To 60 ul of ligations add 100 ul of cells
Incubate on ice 1 hr
Heat 42°C 45 sec
Place on Ice
add 400 ul LB
Incubate 37°C 1 hr.
plate 100 ul on 100 mm plates
300 ul on 150 mm Plates

12/2/94

M15 cells Contaminated? pg 67

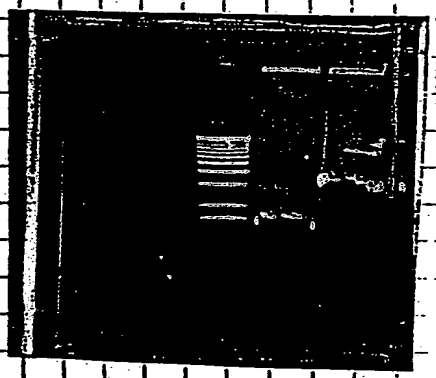
62 | PD10 B/HIII / HTPAN08 Frag / DNaseI frag

11/30/94

Gel isolate fragment on
0.8% LMP gel.

Also Run Ram.
1 HTPAN08 1854bp Bam/Asp
2 HTPAN08 911 bp Bam/Asp
3 DNaseI P Bam/Xba
4 PD10 Bam/HIII
Wash 1 Kb ladder

Run 80V 1½ hrs
Cut out bands & Take Picture



Gene Clean fragments
Add 1ml NaI
Heat 55°C 5 min
Add 5ul Glass milk
Incubate RT 2 min
Spin 10sec
Remove Supernatant
3x { Resuspend pellet in 500ul Wash Buffer
Spin 10sec
Remove Supernatant }

Spin 10 sec
Remove all supernatant possible
Resuspend pellet in 20ul TE for
HTPAN08 51bp + 185bp DNaseI P
Resuspend pellet in 40ul TE PD10 Bam/HIII
Incubate 55°C 2 min
Spin 10sec
Transfer Supernatant to fresh tube.
Re-elute.
Run 2ul on 1% gel with 1Kb ladder
Store at -20°C

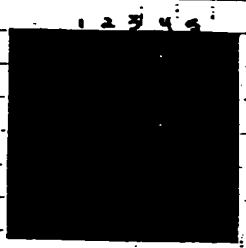
HTPAN08 — see pg 58
DNaseI P - same

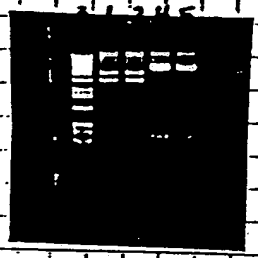
HPRCC91, HE2014Z2

pg60

12/5/94

1. HE2014Z515
2. S14
3. 1kb ladder
4. HPRCC91S14
5. S15





Decided to use a 3' fragment for screening enriched.
Inoculate 150 ml TB + Amp.
HE2014Z S14
HPRCC91S14

12/6/94

Qiagen Maxi prep of: HE2014Z S14
HPRCC91S14

Spin cultures 4.5 K 15 min
Pour off supernatant
Resuspend pellet in 10 ml P1 + RNase
Let sit at RT 5 min
Add 10 ml P2 mix gently
Let sit at RT 5 min
Add 10 ml cold P3 & incubate on ice 20 min
Spin 4.5 K 15 min
Equilibrate Column: 10 ml QBT
Apply supernatant to Column
Wash 2x 30 ml QC
Elute 15 ml QF
PPT with 10.5 ml Isopropanol
Spin 9K 30 min
Pour off Supernatant
Wash 10 ml 70% Ethanol
Spin 9K 10 min
Pour off supernatant

Heidi Pch
12/5/94

HTPA008 504 516p/185bp in PA2

pg 58

12/2/94

The leg Ran alone & MIS cells alone
modified colonies -
Don't use MIS - use DH5α.

Thaw Chemically Competent DH5α
Cells on ice
To 100 ul Cells add 10 ul of
ligation
Incubation ice @ 1 hr
Heat 42°C 45 sec
Put on ice
Add 400 ul LB
Incubate 37°C 1 hr
Plate 200 ul onto 150 mm LB+Amp
Incubate 37°C O/N.

12/3/94

Take plates out of 37°C
Put at 4°C

12/6/94

Pick into 200 ul LB+Amp.
Incubate 37°C a/N w/ rotation

PCR To test for inserts

12/7/94

516bp		40X	185bp		40X
5' Bam 51	0.2	8	5' Bam 185	0.2	4
3' Asp	0.2	8	3' Asp	0.2	4
10X dNTP	3.2	128	10X dNTP	3.2	64
10X PCR	3.2	128	10X PCR	3.2	64
Taq	0.2	8	Taq	0.2	4
H2O	21	840	H2O	21	420
Cult	2		Cult	2	

68 HTPAN08S04 51bp/185bp in PA2.

12/7/92

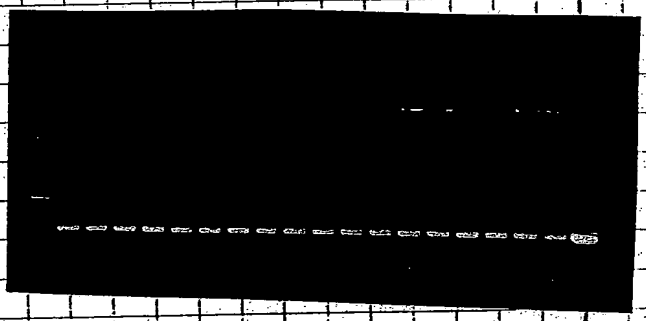
PCR proj # 66.

95°C	5 min	
95°C	20 sec	} 30×
55°C	20 sec	
72°C	1 min	
72°C	7 1/2 min	
4°C	Hold	

Run 10ul on 1% gel with 1Kb Ladder.

HTPAN08S04 51bp

HTPAN08S04 185bp



Pick ⊙ HTPAN08S04 185 bp into ~5 ml TB+Amp Incubate 37°C O/N w/aeration

12/8/94

Boiling mini preps

Spin 2nd cultures 5 min

Remove Supernatant

Resuspend pellet 750 ul STET + Rnase + Lysozyme.

(pg 84)

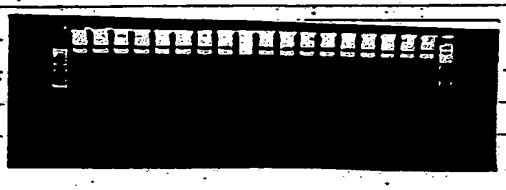
HTPA1508 PA2

PJ68

12/8/94

Boil 1 min
Spin 10 min
Remove pellet
Add 750 ul PEG/NaCl
Mix well
Spin 10 min
Remove supernatant
Add 1000 ul 70% Ethanol
Wash pellet
Spin 5 min
Remove supernatant
Allow pellet to dry at RT 10 min.

Resuspend pellet 20 ul TE
Run 2 ul on gel with 1Kb ladder + λ HIII



Bam with Xba

Try digesting with Bam/Asp.

		19K
DNA	4	
H_2O	22.6	429.4
10x buffer 2	3	57
Bam	0.2	3.6
Asp	0.2	3.6

Incubate 37°C O/N

12/12/94

12/9/94

Does not look like anything digested

Submitt for Sequencing
RP06
RP19.

1/11/95

Remake inserts w/ new primers
for Both PA2 + PD10

PA2 has Bam HI site with
Kozac Seq.

51bp + 185 bp fragments

PCR	③ PD10 HTPAN08 51bp		④ PD10 HTPAN08 185bp		① PA2 HTPAN08 51bp		② PA2 HTPAN08 185bp
9113	20	9114	15	9111	18	9112	15
2742	2	2742	2	2742	2	2742	2
10x PCR	50		50		50		50
10x dNTP	50		50		50		50
Taq	2		2		2		2
H_2O	375		382		377		382
DNA	1		1		1		1

DNA - HTPAN08S04 plasmid DNA - 10ug/ul.

PCR using Program # 66

⊖ control Hrandy

pg 125

PQE60 Nco / Bam Vector

12/19/94

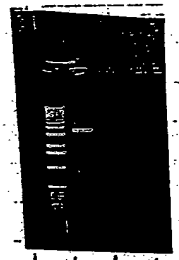
Digest PQE60 = 0.54ug/ul

DNA	4 ul
10x #4	5
H2O	40
Bam	0.5
Nco	0.5
	50

Incubate 37°C O/N

12/20/94

Run 2 ul on gel with 1Kb ladder



looks good.
Add 5 ul 10x SB
Run on 0.8% LMP gel.
80V 1½hr
Cut out fragment.
Take Picture

Gene Clean.
Add 900 ul NaI
Heat 55°C 5 min
mix well.
Add 7 ul Glass milk
Mix well
Incubate at RT 5 min
with occ. mixing
Spin 10 sec
Remove Supernatant
Resuspend pellet 500 ul
3x Wash Buffer
Spin 10 sec

HTPAN08/HTPB411 in PQE60

12/20/

PCR
HTPAN08 S04 5' NcoI / 3' BglII
HTPB411 S15 5' BspHI / BamHI
for ligation into PQE60

HTPAN		HTPB4	
5' NcoI	20	5' BSPHI (5 bp)	20
3' BglII	4	3' Bam	4
10x dNTP	30	10x dNTP	30
10x PCR	30	10x PCR	30
H2O	234	H2O	238
Taq	1	Taq	1
DNA (1ng/ul)	1/300	DNA (1ng/ul)	1/300

Set up Rxn w/o DNA (30 ul vol total)

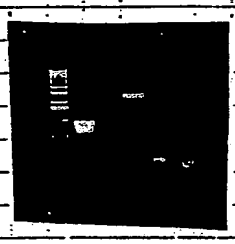
PCR: 2 10x 30 ul Rxns.

Prog 66.

	95°C	5 min
5	95°C	20 sec
5	65°C	20 sec
39 ul	72°C	1 min
0.5	72°C	7 1/2 min
0.5	4°C	hold.
50 ul		

30x

Run 5 ul on gel with 1Kb ladder.

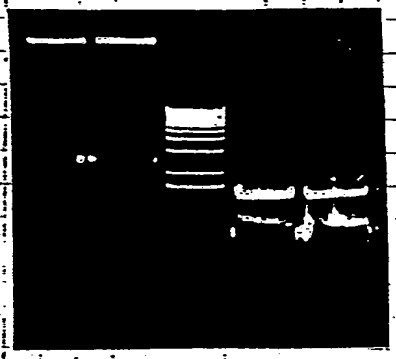


Add equal Vol PEG/NaCl to ppt. - mix well
Spin 10 min
Remove Supernatant
Wash 1X 70% Ethanol (100 ul)
Spin 5 min
Remove supernatant.
Allow pellet to dry at RT 10 min
Resuspend in 10 ul TE

100 HTPA108 | HTPB4H in PQE60

12/20/94

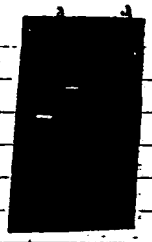
Run 50µl on 0.8% LMP gel
80 V 1½ hrs



Cut out fragments

Spin clean
Resuspend - Total 400µl TE

Run 2µl on gel with 1Kb ladder



looks good -

Set up digestions:

DNA	30µl
H_2O	13
10X #4	5
Enzymes	1/1
	30µl

For HTPA108 - Nco I / Bgl II
HTPB4H Bgl II / Bam -

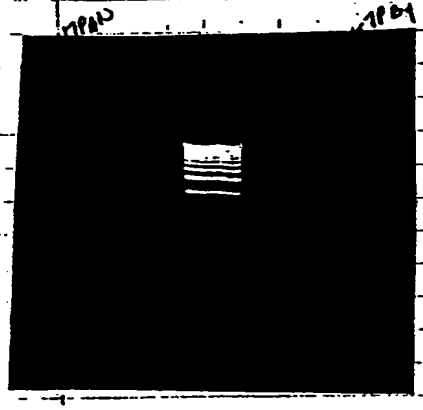
Incubate 37° C O/N.

12/22/94

Add 5µl 10X SB -
Run on 0.8% LMP gel with 1Kb ladder
80 V 1½ hrs

HTPBN08 | HTPB411 in PQE60 | 101

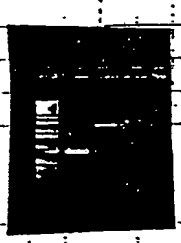
12/22/9



Gene Clean

Resuspend and Elute in 40 ul Total

Run 2 ul on gel with 1Kb ladder



Set up ligation

	①	②	③	④	⑤	⑥
HEPAN08S043'Nco/5'BglII	4	—	4	—		
HTPB411S15 5'Nco/3'Bam	—	4	—	4	—	
10x	2	2	2	2	2	2
H_2O	12	12	13	13	16	1
T4	1	1	1	1	1	1
PQE 60 Nco/Bam	—	1	—	—	—	
PQE 60 Nco/BglII	1	—	—	—		

Incubate 4°C

1/3/95

Transform MIS sup b cells
Chemically competent (Hanahan)

Thaw cells on ice
To 100 ul of cells add 1 ul of ligation
To 1 tube add 10 ul PQE60 DNA
Tube add 10 ul of H_2O only

102 HTRANS08 [HTPSIII in PQE60

1/3/95

Incubate on ice 1 hr
Heat 42°C 1 min
Let sit on ice 2 min
Add 400 µl LB
Incubate 37°C 1 hr.
Plate onto 2 B+ Amp + Kan plates.

1A – 200 µl, 300 µl
1B – 100 µl, 400 µl
2A – 200 µl, 300 µl
2B – 100 µl, 400 µl
3 – 300 µl
4 – 300 µl
5 – 300 µl
6 – 300 µl ligation Rxn.
7 – PQE60 10 ng 100 µl
8 – M15 cells alone 300 µl

Spread Evenly with Beads
Incubate 37° O/N.

1/4/95

All plates grew –
Even M15 cells alone and ligation Rxn alone
Cells are Contaminated!
Told Lang – Said he would test cells and make New.

Try Redoing ligation with
HTRANS504 185 bp fragment.

Pg 107

HTPAN08S04 185 bp Fragment

12/23/9[illegible]

PCR HTPAN08S04 185 bp fragment
for PQE60 Nco/Bgl II

DNA	1 ul	
H_2O	237	
10x PCR	30	Use H_2O only
10mM dNTP	30	do Ⓢ Pipo.
Taq	1	
5' NcoI 185bp	20	
3' Bgl II	4	
	300ul	

Split 10 tubes

PCR Program #66.

95°C	5 min	
95°C	20 sec	30x
55°C	20 sec	
72°C	1 min	
72°C	7 min	
4°C	hold	

Store 4°C

Run
PCR (10 ul with) 1 Kb ladder 1/3/94

Add equal Volume 13% PEG/
1.6M NaCl
incubate on ice 10 min
mixing well
Spin @ 10 min
Pour off supernatant.

104 | HTPAN08S04 | 185bp Fragment

1/3/95

Wash pellet in 700 µl 70% Ethanol.
Spin 5 min.
Pour off supernatant
Allow pellet to dry at RT
Resuspend pellet in 200 µl TE

Now Set up Digestion

DNA	35
H_2O	8
10X #4	5
Nco	1
Bgl II	1
	50 µl

Incubate 37°C O/N.

1/4/95

Run LMP Gel - 0.8% in TAE.
80V 1½ hrs

[redacted]

Cut out fragment from gel and place in Tube
Take picture of gel.

2 Tubes

1 Tube use 6M NaI from Bio 101
Other Tube use 6M NaI made new

Add 1000 µl NaI
Heat 55°C 5 min

(Pg 107)

~~HTL... 180 bp Fragment~~

HTLAD31 PER Protein

1/2/95

PER - Protein

Degest HTLAD31 = 0.25 µg/µl

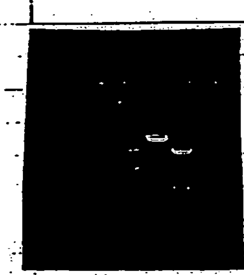
(1) Xho / EcoRI
(2) Sac / Kpn

(1)		(2)	
DNA	6	DNA	6.
H_2O	20.6.	H_2O	20.6
10X #2	3	10X #4	3
Xho	0.2	Kpn	0.2
EcoRI	0.2	Sac	0.2
	30 µl.		30 µl

Incubate 37°C O/N.
Submitt for sequencing -

1/4/95

Run 10 µl in 1% TAE gel
with 1Kb ladder



1- Xho / EcoRI ~4.7 Kb.

2- Kpn / Sac ~800 bp + ~600 bp

Insert looks ~ 1.6 Kb.

Make Primers -
RPO6 & FPO5

1/9/95 Recieved RP06 & RP04

Submit for sequencing with other RP02, RP03 and Reverse & Forward

1/11/95 Recieved FP05.

Submit for Seq RP06 & FP05

1/13/95

Large 3' untranslated region ~1.5 Kb long.

About 1.2 Kb is untranslated.

HTPA1084 SOL 1856p Fragment

(pg 104) & pg 102

1/4/95

Mix well to make sure all of gel is dissolved.
Add 8µl Glass milk & mix.
Incubate at RT 2 min w/ occasional mixing
Spin 10 sec
Pour off supernatant
3x { Resuspend pellet in 400µl Wash Buffer
Spin 10 sec
Pour off supernatant }
Spin 10 sec
Remove as much of Supernatant as possible
Resuspend pellet 30µl TE
Heat 55°C 2 min
Spin 10 sec
Transfer Supernatant to fresh Tube.
Resuspend pellet in 20µl TE
Heat 55°C 2 min
Spin 10 sec
Transfer to tube
Run 2µl on gel with 1 kb ladder

1- using Gene Clean Kit GU Nal

2- using Home made GU Nal

Both look good..
Ready for Ligations

1/5/95

Set up ligation for
HTPA084 HTP8411 in PUC 19

108 HTPAN08 + HTPBY11 in PQE60

1/5/95

	1	2	3	4	5	6	7	8	9
HTPAN08SO4 5'Bbp 5'Nco/3'BglII	6	—	—	6	—	—	—	—	—
HTPAN08SO4 (Bbp 5'Nco/3'BglII	—	6	—	—	6	—	—	—	—
HTPBY11S5 5'BspHI/3'BamHI	—	—	6	—	—	6	—	—	—
10x Buffer	2	2	2	2	2	2	2	2	2
PQE60 Nco/BglII	2	2	—	—	—	—	2	—	—
PQE60 Nco/BamHI	—	—	2	—	—	—	—	2	—
H_2O	9	9	9	11	11	11	15	15	17
T4 DNA Ligase	1	1	1	1	1	1	1	1	—

Make Cocktail

		9x
10X Buffer	2	18
H_2O	9	81
T4 DNA lig	1	9
	12	

Store ligations at 4°C till first M15 cells are found

1/9/95

STEVE said to transform into XL-1 Blue
If plasmid is correct - can transform into M15 cells.

Thaw XL-1 Blue Chemically competent cells on ice
To 10 ul of ligation add 100 ul thawed cells
Incubate on ice 1 hr - as ⊖ control use 100 ul cells only
Heat Shock 42°C 45 sec

P115

Maxi Prep HTPAN08504/HMEAA88

1/4/95

Inoculate 200 ml TB + Amp
from frozen stocks of
HTPAN08504
HMEAA88A.

Incubate 37°C w/ aeration
overnight.

1/5/95

Qiagen Maxi Prep

Spin Cultures 4.5K 15 min
Pour off Supernatant
Resuspend pellet in 10 ml P1 + RNase
Incubate RT 5 min
Add 10 ml P2
Incubate RT 10 min
Add 10 ml P3 - ice cold
Incubate on ice 20 min
Spin 4.5K 15 min
Equilibrate Tip-500 with 10 ml QBT
Apply Supernatant to tip through Kim wipe
allow to flow through
Wash Column 3x
30 ml QC
Elute DNA - 15 ml QF
Add 10.5 ml (add 0.7 times of volumes)
Mix well
Spin 8K 30 min
Pour off Supernatant
Wash pellet 15 ml Cold 70% Ethanol

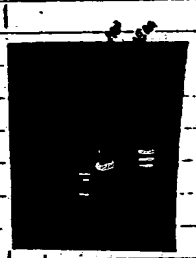
112 Maxi Prep HTRAN08504, HMEAA88

1/5/95

Spin 8K 15min
Pour off supernatant
Allow O/N pellet to dry
Dry O/N at Room Temp -
w/d. parafilm w/holes covering

1/6/95

Resuspend pellet in 400 ul TE
Run 1ul on gel with 1Kb ladder
& λ HindIII Marker



Dilute 1:100 in H_2O
Read $OD_{260/280}$.

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 320.0 nm	280.0 nm / 320.0 nm	
1 [illegible]	0.0402	0.0319	0.0231	1.9374	0.5161	0.2 ug/ml
2 [illegible]	0.2051	0.1314	0.0315	1.7380	0.5754	1.03 ug/ml

looks like HMEAA88 is all chromosomal

1/9/95

Steve said to try transforming with Boiling prep DNA to get a clone

(Pg 108)

1/9/95

Set on ice 2 min
Add 400µl LB.
Incubate 37°C 1½ hrs.
Plate 200µl onto LB+Amp
150mm plates

Transform ligation from 12/22.
400.

Incubate 37°C O/N
Plate M15 Cells on LB+Amp
LB+Kan
LB+Amp/Kan.

1/10/95

Plates look good.
No Contamination

M15 Cells
Grew on LB+Kan
& on LB+Amp+Kan
Not on LB+Amp.
∴ LB+Amp/Kan plates Do not have
Amp or enough amp so contamination
can been seen.

Can Use M15 cells - Not Contaminated

Pick - colonies onto LB+Amp in
96 well dish.

HTPBY11			HTPAN.08.		
12/22	2A	36	12/22	2	48
	2B	44	1/5	1	48
1/5	3	25	1/5	2	48

pick PQE60 Vector + PA2 vector as controls

116 HTPAN08/HTPBU11 in POE60

1/10/95

Incubate 37°C w/aeration 3hrs
PCR using Lentiviral primers
as well as the 2 external Primers

HTPBU11 + POE60	
FP20	0.5
2887	4
2886	0.2
10xdNTP	3.2
10x PCR	3.2
H_2O	18.7
Taq	0.2
Culture	2
	32

HTPAN08 5lbs + POE60	
FP14	0.2
2886	4
2865	0.2
10xdNTP	3.2
10 x PCR	3.2
H_2O	17
Taq	0.2
Culture	2
	32

HTPAN08 185 + PO
FP4 0.2
2867 0.2
2865 0.2
10xdNTP 3.2
10x PCR 3.2
H_2O 28.8
Taq 0.2
Cult 8.0
32

PCR - ① control Plasmid DNA
② control LB + Amp alone

PCR Prog 66

95°C	5min	
95°C	20sec	30X
55°C	20sec	
72°C	1min	
72°C	7min	
4°C	Hold	

Set up Rxns
Let Cultures
grow 37°C O/N
w/aeration

1/11/95

Run 10µl of PCR Rxn on a 1.3% Agarose
gel in TAE with 1Kb ladder

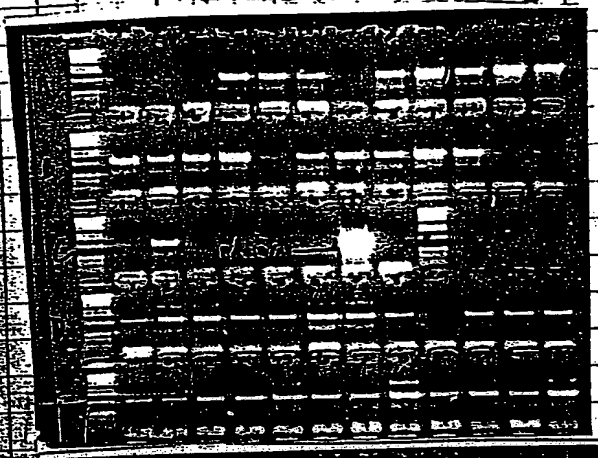
pg 117

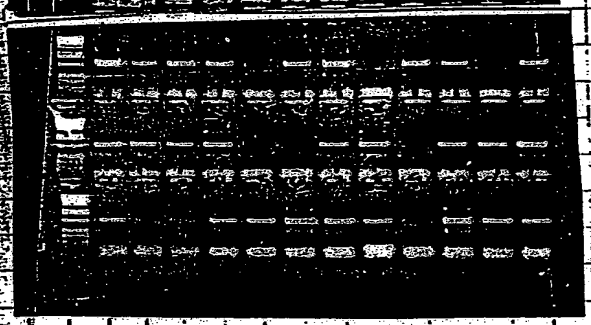
HTRB411 in PAZ / HTPANOS + HTRB411 in PQE60 19

pg 110 pg 116

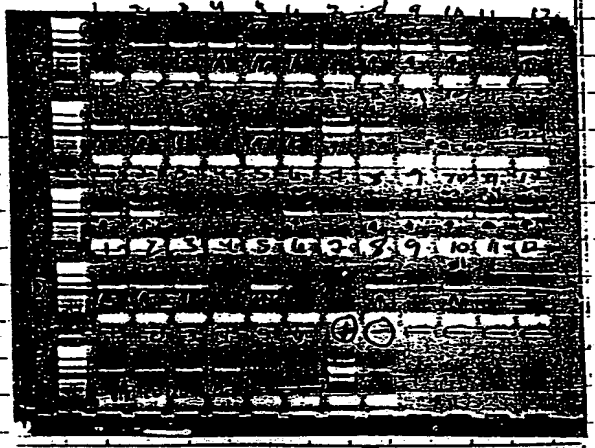
1/11/95

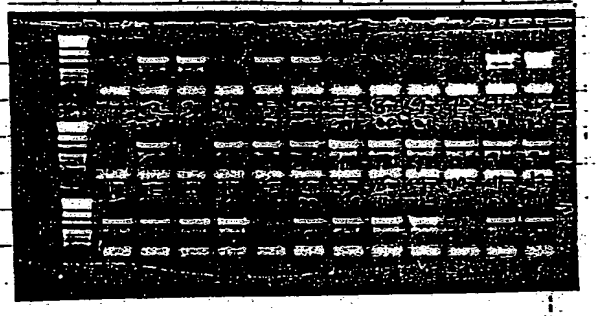
① HTRB411 + PAZ B-E
HTRB411 + PQE60 D-H



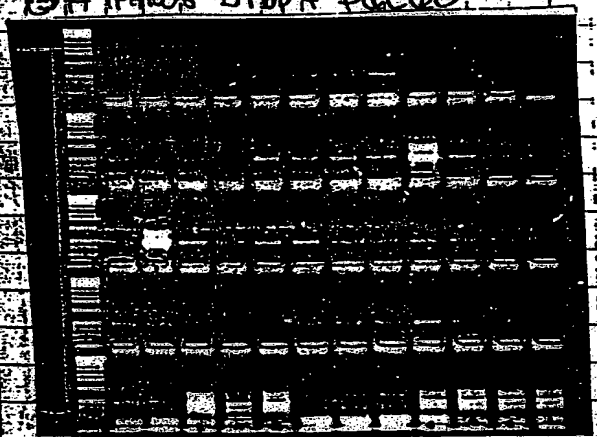


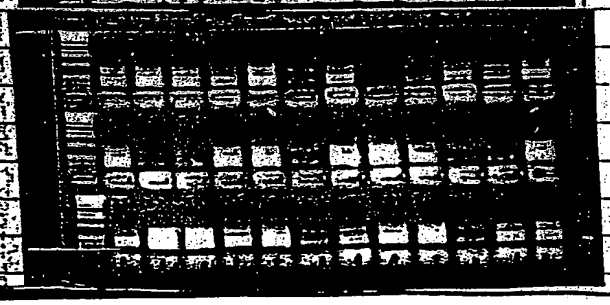
② HTRB411 + PQE60 B-E
HTRB411 + PAZ F-H



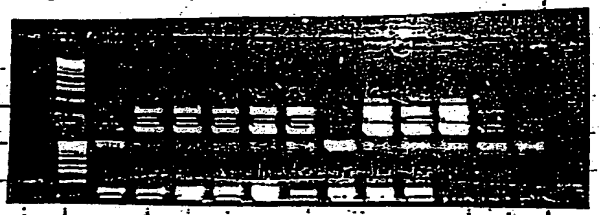


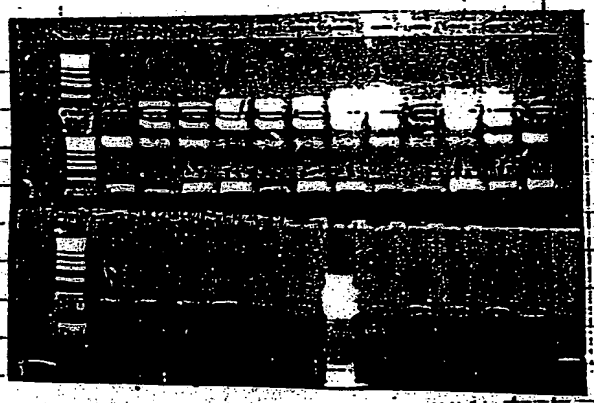
③ HTPANOS 5loop + PQE60





④ HTPANOS 18Sbp + PQE60





120 | HTPB411 PAZ | HTPB411 & HTRAN in PQE60

1/10/95

Inoculate EB TB + Amp 5ml ea
do Boiling preps.

① HTPB411 + PAZ
1, 5, 7, 8, 12, 16, 17, 18, 22, 23, 24, 29, 30, 31, 32, 41, 42, 43

② HTPB411 + PQE60.
2, 6, 7, 19, 23, 24, 29, 38, 41, 45, 48, 57, 58, 66, 69, 73, 74, 79

③ HTRAD085 bp + PQE60.
1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 14, 21, 23, 26, 27, 31, 33, 34

④ HTRAD085 856bp + PQE60
5, 6, 7, 8, 11, 13, 15, 16, 17, 20, 21, 22, 23, 24, 25, 26, 28, 32

Incubate 37°C w/ Aeration overnight

1/12/95

Boiling mini Preps

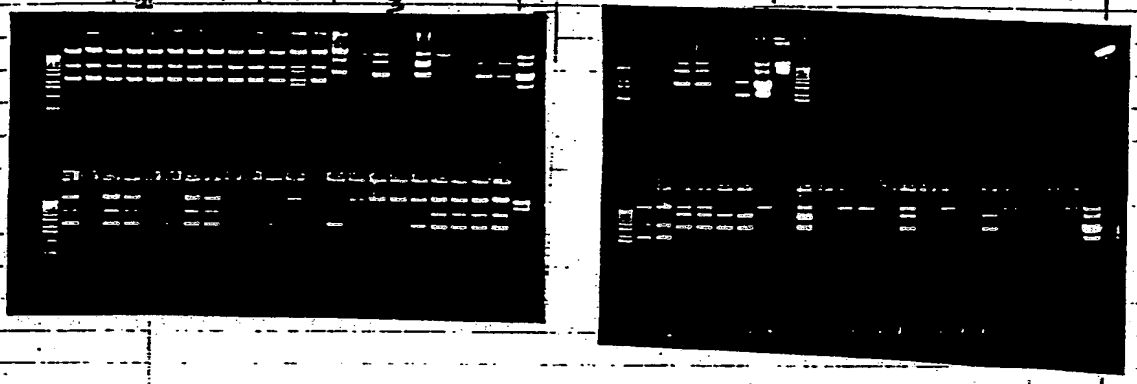
Spin 2ml of culture 2 min
Remove Supernatant
Resuspend pellet in 750 µl STET +
RNase & Lysozyme
Boil 1 min
Spin 10 min
Remove Pellet
Add Equal Volume - 750 µl - of 13% PEG 8000
1.6 M NaCl
Mix Well
Spin 10 min
Remove Supernatant
Wash Pellet 1000 µl 70% Ethanol
Mix

HTPB411 PA2 / HTPB411+HTPAU08 : PQEGO.

4/12/95

Spin 5 min
Remove Ethanol
Allow pellets to Dry at RT for 30 min
Resuspend pellets in 100 ul TE

Run 2 ul of Mini preps on 1% TAE gel with 1Kb ladder + PA2 & PQEGO



mix up in labeling - #1 is really #3
#2 is really #4
#3 is really #1
#4 is really #2

Set up digestions

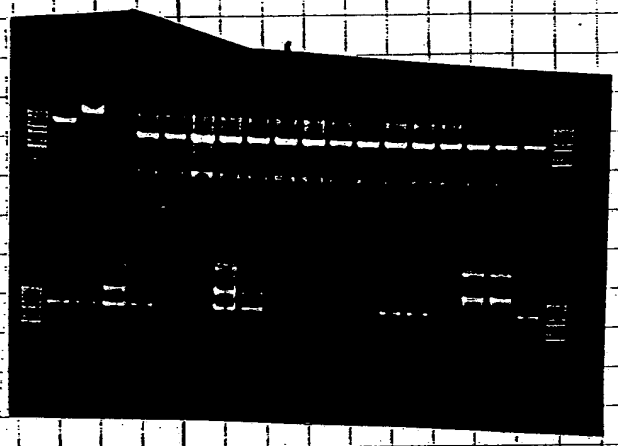
For PQEGO.			For PA2		
EcoRI / HIII		55X	Bam / Xba		20X
DNA	4	—	DNA	4	—
10X	2	110	10X	2	40
H_2O	13.6	748	H_2O	13.6	272
EcoRI	0.2	11	Bam	0.2	4
HIII	0.2	11	Xba	0.2	4

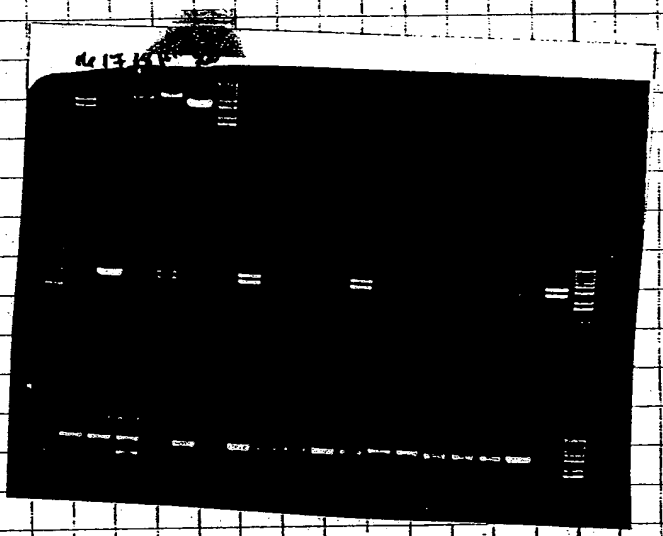
Incubate 37°C O/N
Digest HTPAU0804 HTPB411515
PA2 & PQEGO

122 HTPB4H PR2 / HTPAN08 + HTPB4H PCR'd

1/13/95 1,2,4 - Eco / HIII 3, Bam / Xba digested

Run 10ul on gel with 1Kb ladder





Looks like
4 - HTPAN08 185bp + PCR'd
1 - 17 correct - Sequence 4.
2. HTPB4H in PCR'd
1-4, 6, 8 - 16 looks good Seq 4.
1 + 3 Need to be digested w/ correct Enzymes

Submit 4 + 2 (4 of each)
#4 with RP016 & FP16
#2 FP23
for Sequencing

1/16/95

Clean up DNA of 4-1, 4-2, 4-3, 4-4
2-1, 2-2, 2-3, 2-4
2x Phenol
2x CH Cl3 : IAA
Ethanol PPT - Wash
Resuspend in 80 ul TE - use to transform into M15 cells

pg 127

HTPAN08 PAZ/PDIO

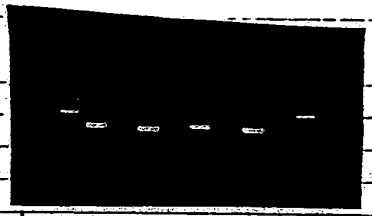
pg10

95°C 5min
95°C 20sec
55°C 20sec
72°C 1min
72°C 7.5min
94°C Hold

30x

Run 5µl on gel with 1kb ladder 11/12/95

3 - HTPAN08 351 bp for PAZ
4 - HTPAN08 185 bp for PAZ
1 - HTPAN08 351 bp for PDIO
2 - HTPAN08 185 bp for PDIO



4/5ths

1

Precipitate, Add equal Volume
3% PEG/NaCl
Spin 10 min
Wash pellet 1000µl @ 70% Ethanol
Spin 5 min
Remove Supernatant
Set up digestions

DNA (PCR Fragment)	20
10x #2 Buffer	4
H_2O	15
Bam	0.5
Xba	0.5
	40µl

Incubate 37°C Overnight

126 HTPAN08 PAZ / PDIO

1/13/95

Run All of digestion of 0.8% Agarose LMP Gel

Cut out gel fragments.
Take picture

1 - HTPAN0851bp > PDIO
2 - HTPAN08185bp
3 - HTPAN0851bp > PAZ.
4 HTPAN08185bp

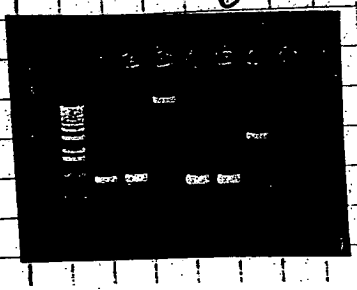
Gene Clean
Add 800 ul NaI
Heat 55°C 5min
Add 8 ul Glass Milk
Mix well
Incubate At RT 5min
w/ occasional mixing
Spin 10sec
Remove Supernatant

3X [Add 500 ul Wash Buffer
Resuspend pellet
Spin 10sec
Remove Supernatant]

Spin 10sec
Remove Supernatant
Supernatant
Resuspend pellet in 20 ul TE

2X [Heat 55°C 1hr
Spin 10sec
Transfer Supernatant to fresh tube]

Run on gel with 1/10 loading and 1 ul of PAZ or 1 ul PDIO B/X



1 HTPAN08 51bp PAZ
2 HTPAN08 185bp PAZ
3 PAZ Bam 1hr
4 HTPAN08 51bp PDIO
5 HTPAN08 185bp PDIO
6 PDIO Bam 1hr

HTPAN08/HTPB41 PAZ/PD10

pg 122

1/13/95

Set-up ligations

	1	2	3	4	5	6	7	8	9	10	11	12	13
HTPAN08 SI PAZ	6	—	—	—	—	6	—	—	—	—	—	—	—
HTPAN08 SS PAZ	—	6	—	—	—	—	6	—	—	—	—	—	—
HTPAN08 SI PD10	—	—	6	—	—	—	—	6	—	—	—	—	—
HTPAN08 SS PD10	—	—	—	6	—	—	—	—	6	—	—	—	—
HTPB41 PD10	—	—	—	—	6	—	—	—	—	6	—	—	—
10X Buffer	2	2	2	2	2	2	2	2	2	2	2	2	2
H_2O	9	9	9	9	9	11	11	11	11	11	15	15	17
T4 Ligase 1U/µl	1	1	1	1	1	1	1	1	1	1	1	1	1
PAZ Bam/Xba	2	2	—	—	—	—	—	—	—	—	2	—	—
PD10 Bam/Xba	—	—	2	2	2	—	—	—	—	—	—	2	—

Set up ligations on ice
Incubate 16°C over the weekend

over

1/14/95

Thaw MI5 chemically competent cells on ice
To 100µl of Cells add 10µl of ligation (from 1/13/95) and 1µl of DNA (from pg 122)
Do Tube of MI5 cells only / 1 of 10ng PD10
Incubate on ice 1 hr
Heat 42°C 45sec
Place on ice
Add 400µl LB
Incubate 37°C 1½ hrs
Plate onto LB + Amp 150mm plates
- ligations 1-5 plate 200+300µl
- Vector DNA plate 50µl
- Ligation Controls 1-3 plate 300µl
- MI5 cells plate 300µl
- MI5 cells +PD10 plate 100µl

Incubate 37°C O/N

1/16/95

HTPBY11+PA2
1/10/95 Transformation
Pick 96 colonies into LB+Amp

HTPAN08 51bp + PQE60
1/10/95 Transformation
48 from 1/5/95 ligation
48 from 12/22/94 ligation
into 200ul LB+Amp
Incubate 37°C w/ aeration O/N.

1/17/95

Transformations worked well -
No Colonies seen on fragment only Plate
2-3 colonies seen on Vector alone plate.
No Colonies on - M15 cells alone
- Lig Rxn alone.

Pick colonies into 200ul LB+Amp+Kan

Plate [1] (1) HTPAN08 51bp+PA2 - 48 A1→D12
(2) HTPAN08 185bp +PA2 48 E1→H12

[2] (3) HTPAN08 51bp +PD10 - 48 A1→D12
(4) HTPAN08 185bp +PD10 - 48 E1→H12

[3] (5) HTPBY11 + PD10 - 16 A1→B4
PDY0 4
PA2 4

[4] 2-1 (12) } HTPBY11 + PQE60 A1-A12
2-2 (3) } B1-B3
2-3 (12) } in M15 cells C1-C12
2-4 (12) D1-

HTPB411 + HTPAN058

1/17/95

E1-E12 4-1 (12)
F1-F12 4-2 (13)
G1-G12 4-3 (14)
H1-H12 4-4 (15)
} HTPAN058 185bp + PQE60 M13 cells.

Incubate @ 37°C w/aeration 4 hrs.

PCR - HTPB411+PA2 & HTPAN058 51bp + PQE60

HTPAN058 51bp PQE60.

		100X
2888	2	200
7409 FP16	0.15	15
10x dNTP	3.2	320
10x PCR	3.2	320
H_2O	21.25	2125
Taq	0.2	20
Cult	2	
	32ul	30ul/tube

HTPB411 + PA2 100X

2741	0.2	20
7796 RPCR	1.2	120
10x dNTP	3.2	320
10x PCR	3.2	320
H_2O	22	2200
Taq	0.2	20
Cult	2	
	32ul	30ul/tube

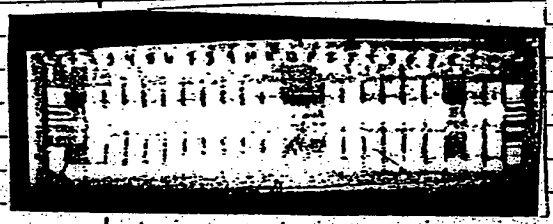
PCR Prog C06.

95°C	5min	
95°C	20sec	30x
55°C	20sec	
72°C	1min	
72°C	7 1/2 min	
4°C	Hold	

Run Qiaken gel with 1 Kb ladder

☺ control use 1B Vaccinia only

1→D12
1→H12
7D12
2H12
7B4
-A12
-B3
-D12
-D12



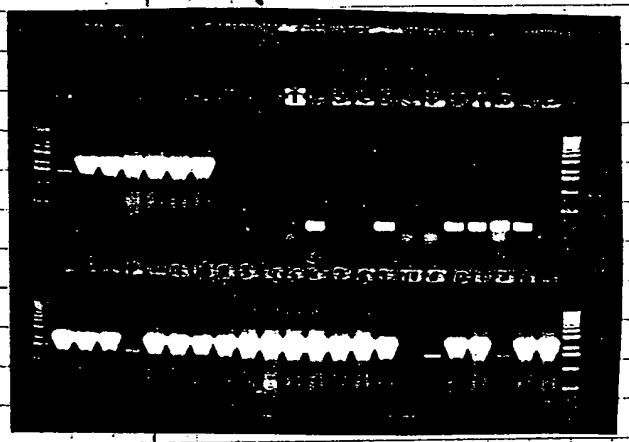
HTPB411+PA2
2741 (3'Vbar) + 7796 (RPCR)

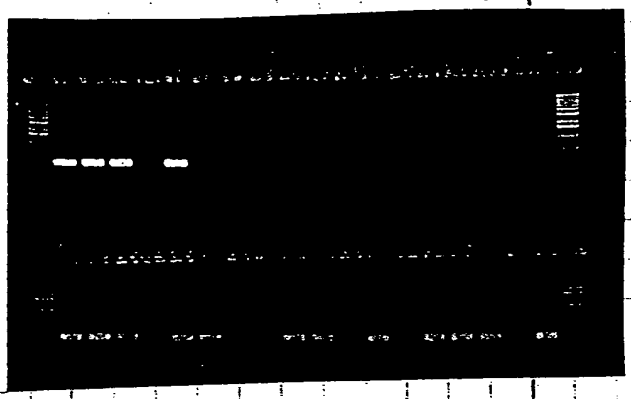
130

HTPB411 & HTPAN08

1/17/95

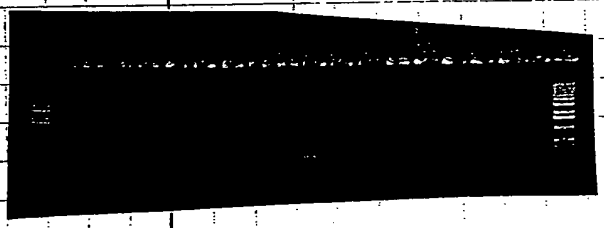
[#7PAN08 5'bsp + PQE60 (2888 5'bsp #7 + #409 (FP11e)

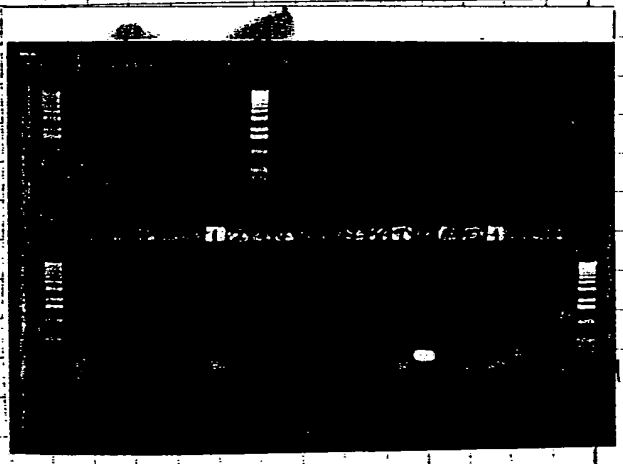




↑

HTPB411 + PA2
2741 (3'xba2) + 7796 (RPL6)





HTPAN08 51bp + PQE60
2888 (5'BspHI) +
7409 (FP11e)

Inoculate 5ml TBT Amp w. tn.
@ clones 1-9 from HTPB411 + PA2
HTPAN08 51bp + PQE60

Incubate 37°C w/ aeration O/N

PCR Plates [1], [2], [3], [4]

HTPBHII & HTPAN08

1/17/95

Plate 1

① HTPAN08 518bp + PA2

		55X
9111	1.7	66
2742	0.1	5.5
10xdNTP	3.2	176
10xPCR	3.2	176
H_2O	22.1	1215.5
Taq	0.2	11
Cult.	2	
	32 µl	32µl/Tube

+control HTPBHII

② HTPAN08 185bp + PA2

		55X
9112	1.7	66
2742	0.1	5.5
10xdNTP	3.2	176
10xPCR	3.2	176
H_2O	22.1	1215.5
Taq	0.2	11
Cult.	2	
	32µl	30µl/Tube

③ Control PA2 Vector & LB Amp/Kan @ Control HTPAN

Plate 2

③ HTPAN08 518bp + PD10

		55X
9113	1.4	77
2742	0.1	5.5
10xdNTP	3.2	176
10xPCR	3.2	176
H_2O	21.9	1204.5
Taq	0.2	11
Cult	2	
@ control HTPAN	32	30µl/Tube

④ HTPAN08 185bp + PD10

		55X
9114	1.2	66
2742	0.1	5.5
10xdNTP	3.2	176
10xPCR	3.2	176
H_2O	22.1	1215.5
PBAD Taq	0.2	11
Cult	2	
	32	30µl/Tube

⑤ Control PD10 Vector + LB Amp/Kan @ Control HTPAN

Plate 3

⑤ HTPBHII + PD10

		24X
2752	1.2	28.8
2741	0.2	4.8
10xdNTP	3.2	76.8
10xPCR	3.2	76.8
H_2O	22	528
Taq	0.2	4.8
Cult.	2	
	32µl	30µl/Tube

⑥ control PD10 Vector + LB Amp Kan @ Control HTPBHII

HTPB411 + HTPAN08

1/17/95

Plate 4

① HTPB411 + PQE60		50x
29867	3	150
29856	0.2	10
10xdUTP	3.2	160
10xPCR	3.2	160
H_2O	20.2	1010
Taq	0.2	10
Cult	2	
	32	32µl/Tube

② HTPAN08185 + PQE60		50x
29867	0.2	10
29805	0.2	10
10xdNTP	3.2	160
10xPCR	3.2	160
H_2O	23	1150
Taq	0.2	10
Cult	2	
	32	32µl/Tube

③ Control HTPB411

④ Control HTPAN08

⑤ Control LB2 Amp/Kan

PCR Prog 66.

95°C	5min	
65°C	20sec	30x
55°C	20sec	
72°C	1min	
92°C	7½min	
4°C	hold	

Run 10µl on gel with 1K bladder.

Run by plate.

Plate 3

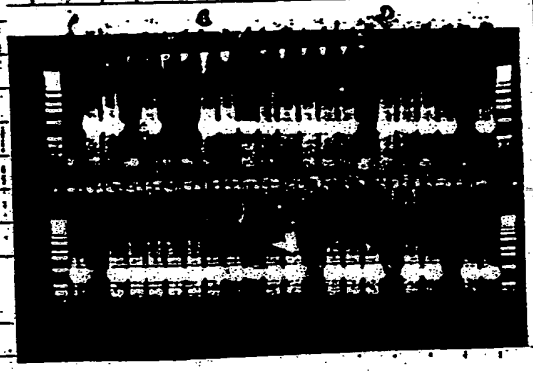
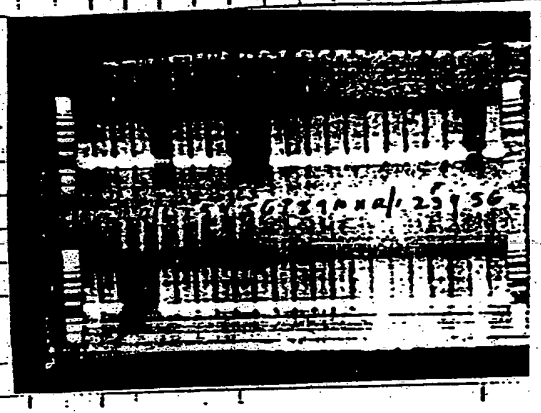


Plate 5



HTPB411 & HTPAN

11/17/95

Plate 1 + Plate 2

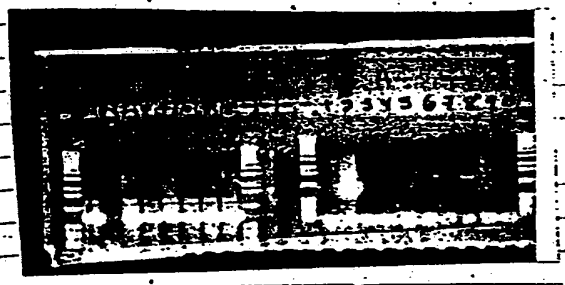


Plate 2

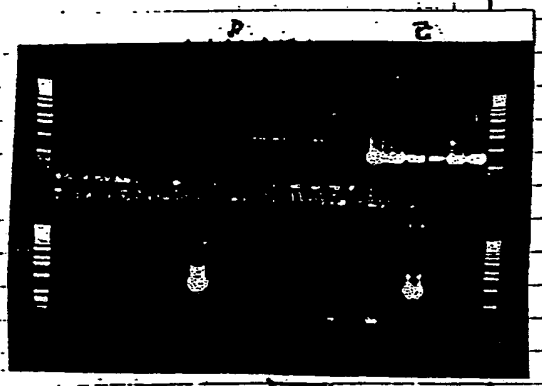


Plate 2

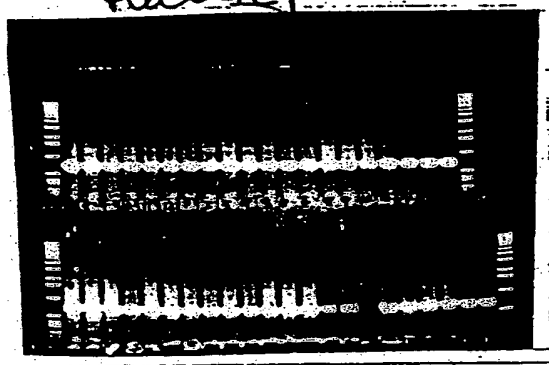


Plate 3

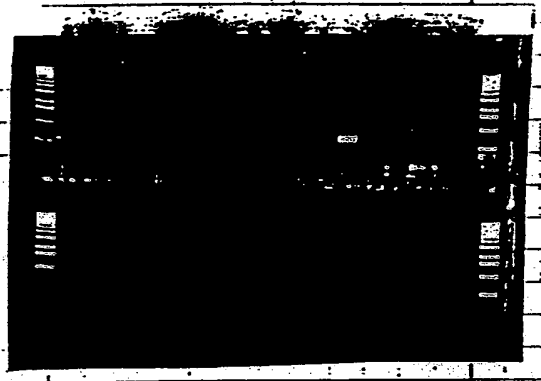


Plate 5 Plate 4

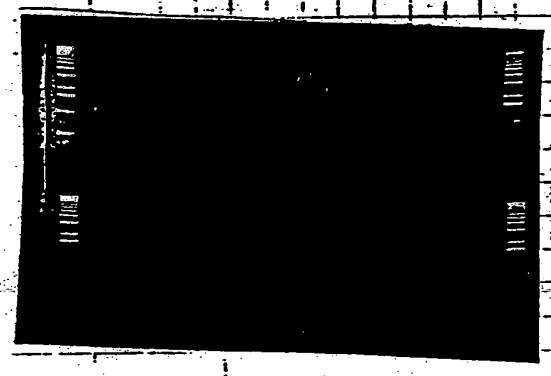
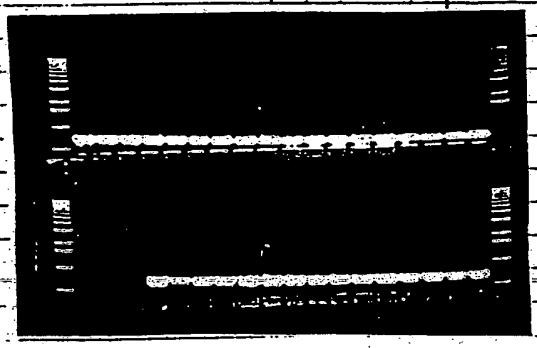


Plate 4

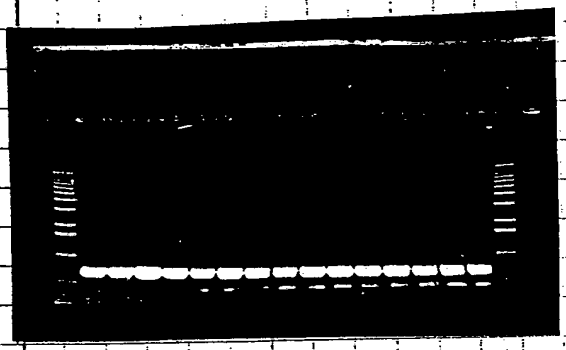


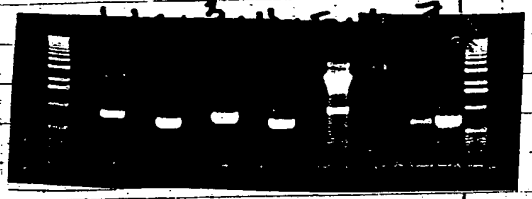
HTPAN08 ~ HTPB411

1/17/95

Plate 4

Controls





Controls

①	9111 + 2742	HTPAN08	51bp PAZ
②	9112 + 2742	HTPAN08	185bp PAZ
③	9113 + 2742	↓	51bp PD10
④	9114 + 2742	↓	185bp PD10
⑤	2752 + 2741	HTPB411	PD10
⑥	2887 + 2886	HTPB411	PQE60
⑦	2967 + 2865	HTPAN08	185bp PQE60

Inoculate 5ml LB + Kan/Amp
~~with 2 clones ea~~
with 2 clones each of ④-1, ④-2, ④-3, ④-4 (A+B)
HTPB411 + PQE60 - ⑥-1, ⑥-2, ⑥-3, ⑥-4 (A+B)
HTPAN08 185bp + PQE60: ⑦-1, ⑦-2, ⑦-3, ⑦-4 (A+B)

Inoculate 5ml TB + Amp
with 9 clones of each.

HTPAN08	51bp + PAZ	- 9
HTPAN08	185bp + PAZ	- 9
HTPAN08	51bp + PD10	- 3
HTPAN08	185bp + PD10	- 9
~~HTPB411~~	~~PQE60~~	
HTPB411	PD10	- 9

Incubate 37°C O/N w/ aeration

HTPB411 & HTPAN084

11/18/95

HTPB411 + PQE60.
HTPAN084 185bp + PQE60.

To 5ml LB + Amp/Kan add 200µl of ON Cultures.
Incubate 37°C w/ aeration till OD_{600} = 0.4 - 0.6. - 2hrs
Add 100mM IPTG to 2mM → 110µl.
Incubate 37°C 4hrs.
Spin 750µl of culture - induced and ① of uninduced of HTPAN084 HTPB411.
Remove supernatant
Resuspend pellet in 100µl H_2O
Add 100µl 2x dissociation Buffer.

2x dissociation Buffer:
- 0.125M Tris pH 6.9
- 4% SDS
- 20% Glycerol
- 10% β-Mercapto Ethanol
- 0.2% Bromophenol Blue

Mix well
Heat 100°C 5min
Spin 5min
Put on ice
Run 10µl on 12.5% Acrylamide stacking gel with Rainbow marker
Run at 150V in 1x Running Buffer for 1½ hrs. Tell dye front is at bottom of gel
Stain - ½ hr at 37°C
De Stain overnight at RT -

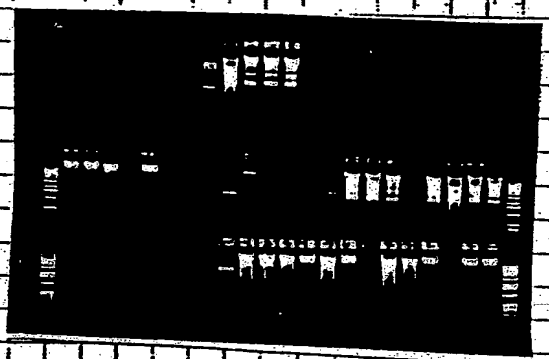
Boiling Mini preps.

HTPB411 & HTPAN08

4/18/95

HTPB411 + PAZ
HTPAN08 51bp PAZ
HTPAN08 185bp PAZ
HTPAN08 51bp PDIO
HTPAN08 185bp PDIO
HTPAN08 51bp PQE
} Boiling minis.

Some cultures did not grow.
Spin 2ml cultures
Remove supernatant
Resuspend pellet in 750µl STET + RNase/Lysozyme.
Boil 1 min
Spin 10 min
Remove pellet
Add 750µl 13% PEG 8000 / 1.6M NaCl
Mix well
Spin 10 min
Remove supernatant
Wash pellet 1000µl 70% Ethanol
Spin 5 min
Remove supernatant
Allow pellet to dry at RT 30 min.
Resuspend pellet in 150µl TE
Run 2µl on gel with 1Kb ladder



A = HTPB411 + PAZ
B = HTPAN0851 + PAZ
C = HTPAN08185 + PAZ
D = HTPAN0851 + PQE
E = HTPAN08185 + PDIO
F = HTPAN0851 + PDIO

Set up digestions

HTPBγII & HTPAD08.

1/18/94.

for PAZ + PD10 Constructs
Digest: BamHI / XbaI

		40X
DNA	5	—
10x#2	3	120
H_2O	21.6	874
BamHI	0.2	8
XbaI	0.2	8
	30µl	25µl/tube

For PQE Constructs
digest EcoRI / HindIII

		10X
DNA	5	—
HDX#2	3	30
H_2O	21.6	216
EcoRI	0.2	2
HindIII	0.2	2
	30µl	25/tube

Incubate all reactions at 37°C O/N.

1/19/95.

Digestions of Mini Preps:

Run 10µl of Digestion on 1% TAE Agarose gel with 1Kb ladder

A HTPBγII + PAZ
B HTPAD08 518bp + PAZ
C 185bp + PAZ
D 518bp + PD10
E 185bp + PD10
F 518bp + PQE60

Looks like some digested correctly

Clean-up and send on to be sequenced with internal primers.

HTPAN08 / HTPB411

1/19/95

Dilute HTPB411 + PA2 1,2,3,4
HTPAN08 516p + PA2 1,2,3,4
HTPAN08 1856p + PA2 1,3,4,5.

1:200 - use 10ul to transform into DH5α.

Thaw DH5α Chemically Competent cells on ice
To 100ul of thawed cells Add 10ul of Diluted DNA.
Incubate on ice 1hr
Heat 42°C 45sec
place on ice
Add 400ul LB
Incubate 37°C 1hr.
Plate 300ul onto 150mm LB+Amp plates.
Incubate 37°C O/N.

PCR HTPB411 + PA2
HTPAN08 516p + PA2
HTPAN08 985p + PA2
With a PA2 specific T7 primer and the 3' end primer

HTPAN		HTPB411	
T7	0.2	T7	0.2
2742	0.2	2741	0.2
10X	10	10X	10
10X	10	10X	10
H_2O	78.2	H_2O	78.2
Taq	0.4	Taq	0.4
DNA	1 ul of Diluted	DNA	1 of diluted
	100ul		100ul

4 Tubes of each

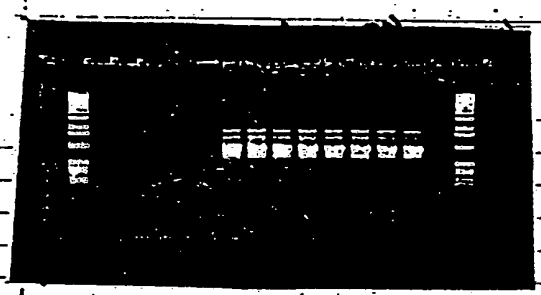
HTPAN08 / HTPB411

1/19/95

Run Program #66.

95°C	5min	
95°C	30sec	30x
55°C	20sec	
72°C	1 min	
72°C	7 1/2 min	
4°C	Hold.	

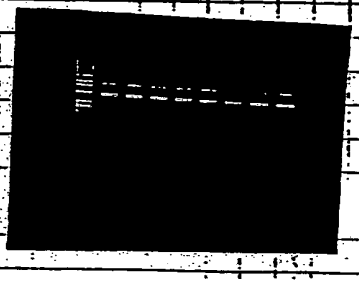
Run 5ul of PCR on 1% TAE gel with 1Kb ladder.



HTPB411 Did Not PCR - Need to get More P Clones.

HTPAN08 looks good.

HTPAN08 - precipitate with equal Vol 13% PEG / 1.6M NaCl
Mix Well
Spin 10min
Pour off Supernatant
Wash pellet 1ml 70% Ethanol
Spin 5min
Pour off supernatant
Allow pellet to dry at RT 15min.
Resuspend in 100 µl TE
Run 2ul on 1% gel with 1Kb Ladder



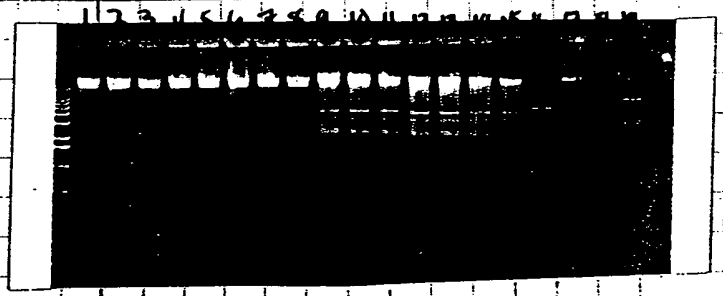
Don't know about upper band ~2Kb - but band looks good
Do TNT 1/20.

140 HTPAN08 / HTPD911

1/19/95

Clean up Boiling preps to submit for sequencing

Add TE to QS all samples to 400ul.
2x Phenol / SEVAG (1:1)
Extract with Equal Volume
2x SEVAG Extract with Equal Volume
Add 1/10 Vol (40ul) 3M NaOAc pH 5.3
2 Vol (~900ul) 100% Ethanol
Mix well
Set on ice 10 min
Spin 15 min
Remove Supernatant
Wash pellet 1000ul 70% Ethanol
Spin 5 min
Remove supernatant
allow pellet to dry at RT 5 min.
Resuspend pellet in 100ul TE.
Run Sulfon gel with 1kbladder



(A) 1-4 HTPAN08 51bp+ PAZ
(B) 5-8 HTPAN08 185bp+ PAZ
(C) 9-12 HTPAN08 51bp+ PDJO
(D) 13-16 HTPAN08 185bp+ PDJO
(E) 17-19 HTPAN08 51bp+ POLCO

Submit for Sequencing FP16 + RPO6.

IRIS Names:
(A) FAS51BP1PAZRPO6/F
(B) FAS185B1PAZRPO6/RP1
(C) FAS51BP1PDJRPO6/FP1
(D) FAS185B1PDJRPO6/FP1
(E) FAS51BP1POLRPO6/FP16

1+TPANO8 / HTPBU11

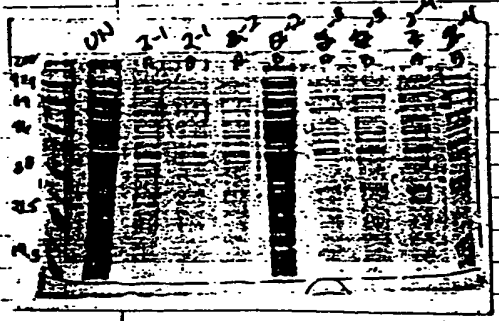
1/29/95

For
Inoculate 5ml LBT Amps (Kan
with HTPBU11+pQE60
HTPANO8 185 +pQE Constructs

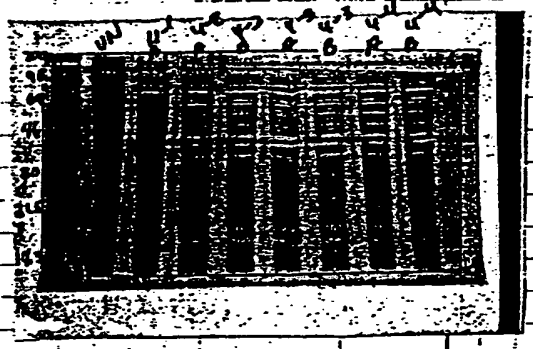
pg 135 –

Destained gel

HTPBU11+pQE60



HTPANO8 185bp +pQE60



Res more of Sample on gel – 10%.

Stain – Destain

looks like #4 reduced

1/29/95

Can't really tell. Try inducing more

142 HCPB411+HTPB1005

1/20/95 DO TNT with PCR'd T7 fragments (pg 139)

Rabbit Reticulocyte Lysate	12.5
T7 Buffer	1
T7 Polymerase	0.5
Amino Acid -Met	0.5
^{35}S methionine	2
RNasin	0.5
PCR Product	4
H_2O	4
	25 ul

Incubate 30°C 2 hours
Heat 90°C 5 min
Quick Spin
To 20ul of 2X dissociation buffer
Add 5ul of TNT

Run 15ul on 15% Acrylamide gel with C14 layed Rainbow marker
Run gel 150V 1½ hrs
until Dye front is almost to bottom.
Cut off stacking gel
Fix gel in 1 Fixative
10% HoAc; 30% MeOH
for 20 min at 37°C
Pour off fix
Amplify in 30 ml Amplify
for 20 min at 37°C
Dry gel 1½ hrs at 80°C.
Place on cellophane
Leave at RT over weekend

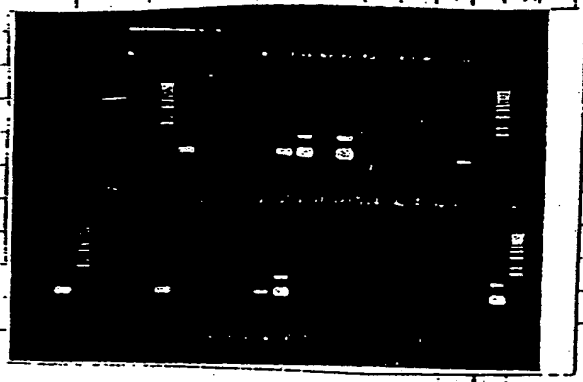
1/22/95 Inoculate 15ml TBtAmp / TBtAmp Kan
with cultures / 37°C O/N

pg 149

IL-6 PQE66 / PD10

pg 70

1/19/95



IL-6 PD10

Looks like Correct Clones

IL-6 PQE					IL-6 PD10				
1-A2	6 B10	11 D12	14 G5		1-A2	6-C4	11-E5	16 G1	
2-A3	7 C1	12 E5	17 G8		2-A7	7-C5	12 E11	17 H2	
3-A7	8 C4	13 E8	18 H5		3-B1	8-C8	13 F3	18 H3	
4-A10	9 C9	14 E11	19 H8		4-B4	9-C9	14 F9	19 H5	
5-A11	10 D10	15 F1	20 F10 ?		5-B11	10-D7	15 G8	20 H7	

1/23/95

Inoculate 200ml LB + Amp/Kan with Cultures 1-20 of each. Incubate o/N at 37°C w/ aeration to Do Mini inductions

1/24/95

To 200 ml of fresh LB + Amp/Kan add 50 µl of fresh Cultures

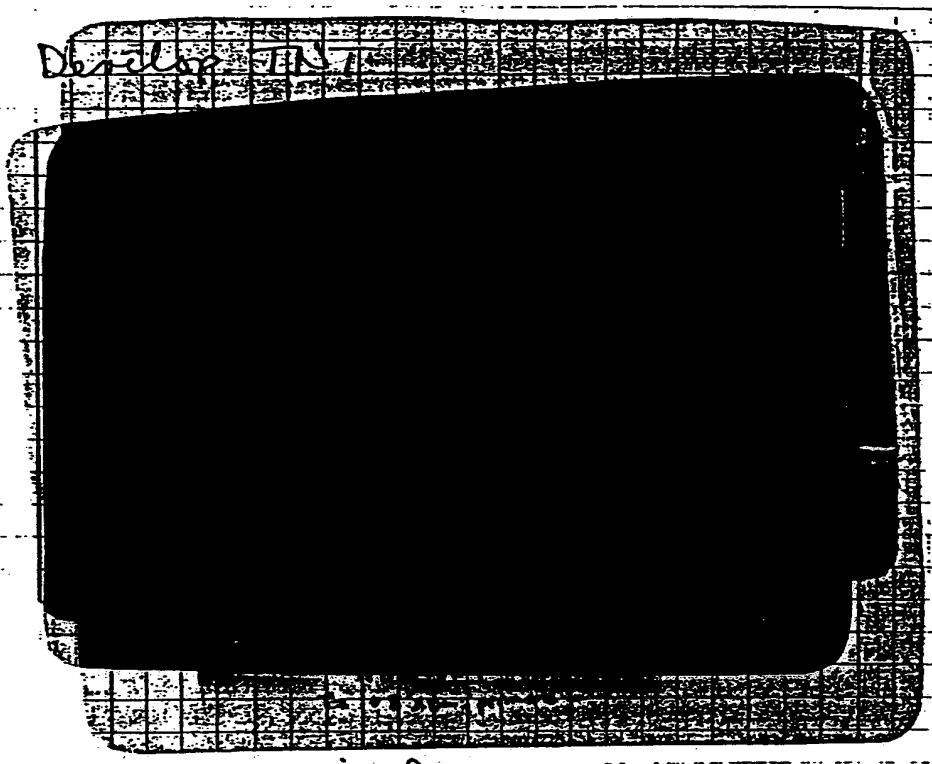
HTPB411 + HTPAN08

149

Pg 42

1/23/95

Develop [redacted]



Qiagen Maxi Preps of:

HMEAA88
HTPAN08504 518p + PA2 #1
+ PA2 #3
+ PA2 #4
185bp + PA2 #1
+ PA2 #3
+ PA2 #4
+ PA2 #5
+ PQE60 4-1
+ PQE60 4-2

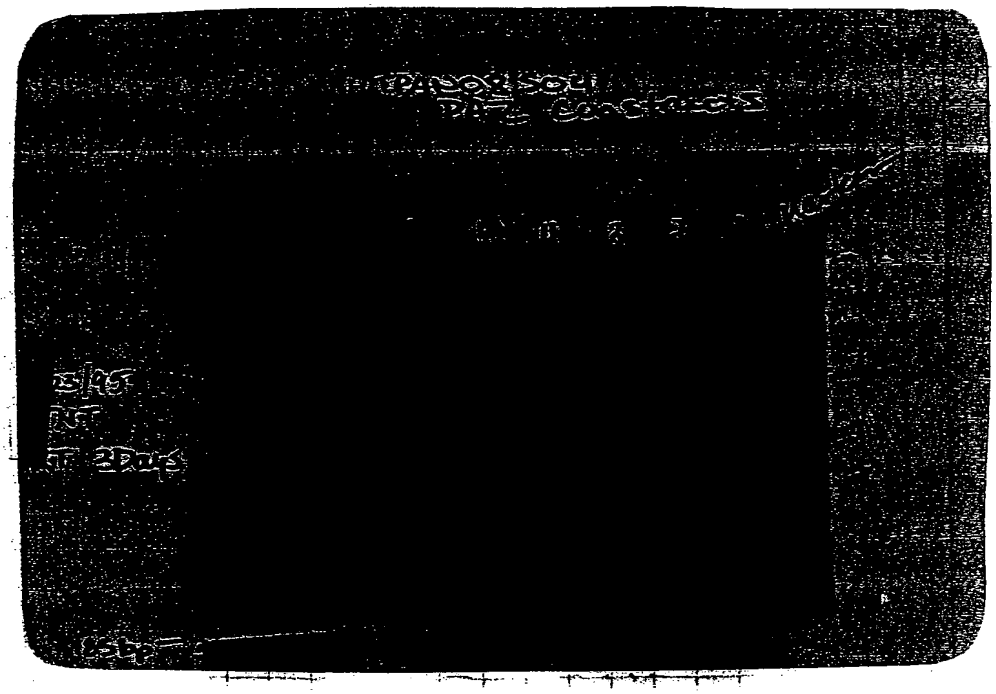
HTPB411515 + PA2 1

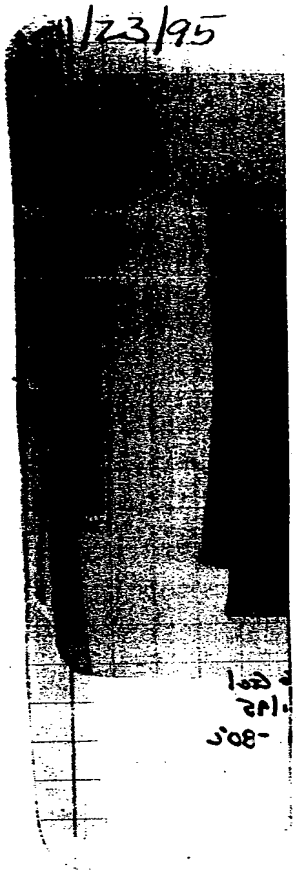
Make Glycerol stocks of all
800ul Bacterial Stock + 800ul 30% Glycerol

HTPB411 + HTPAN08

Pg 42

Develop TNT.





Qiagen Maxi Preps of:

HMEAA88
HTPAN08S04 518p + PA2 #1
+ PA2 #3
+ PA2 #4
185bp + PA2 #1
+ PA2 #3
+ PA2 #4
+ PA2 #5
+ PQE60 4-1
+ PQE60 4-2

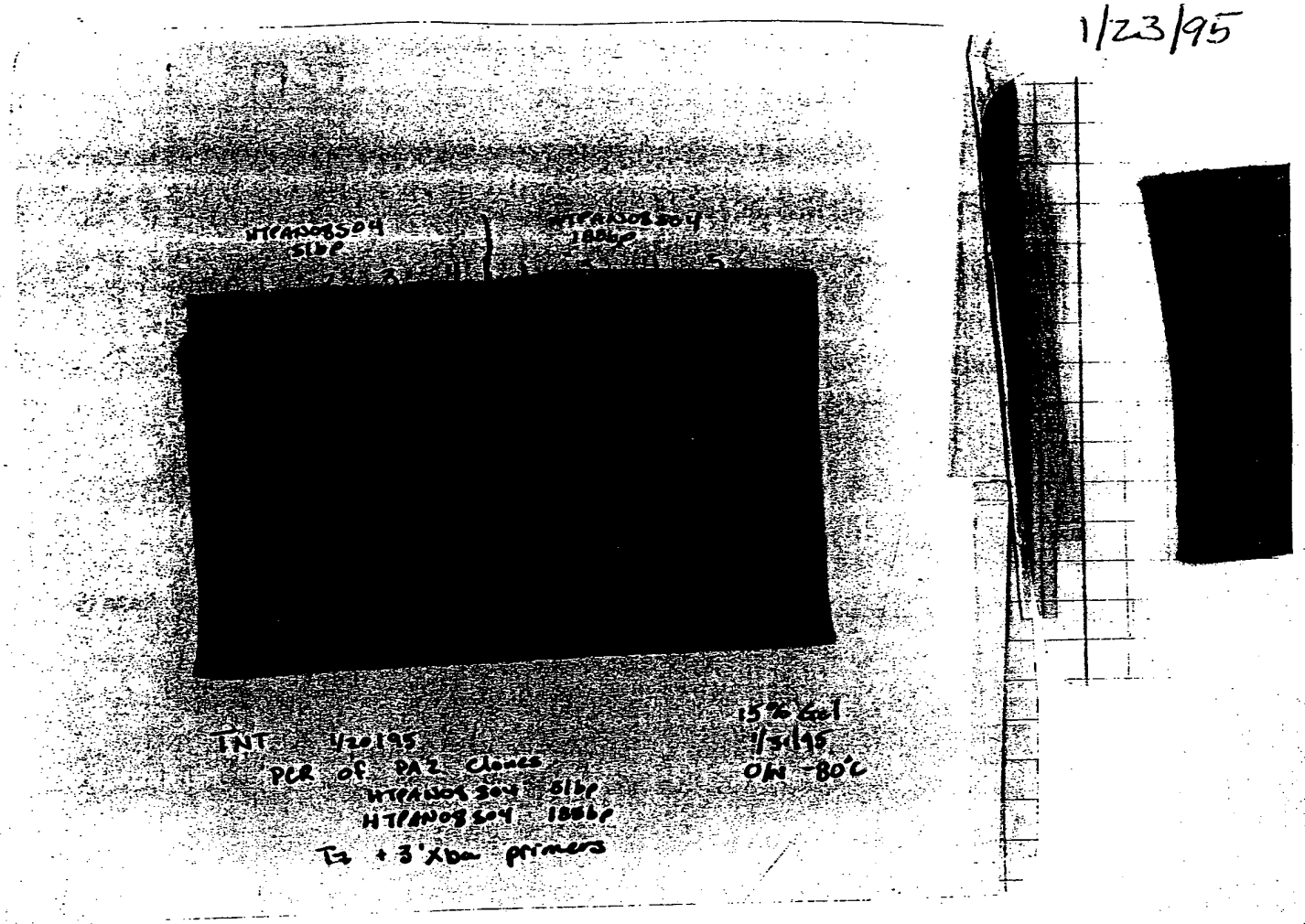
HTPB411S15 + PA2 1

Make Glycerol stocks of all
800ul Bacterial Stock + 800ul 30% Glycerol

HTPB411 + HTPAN08

pg 42

1/23/95



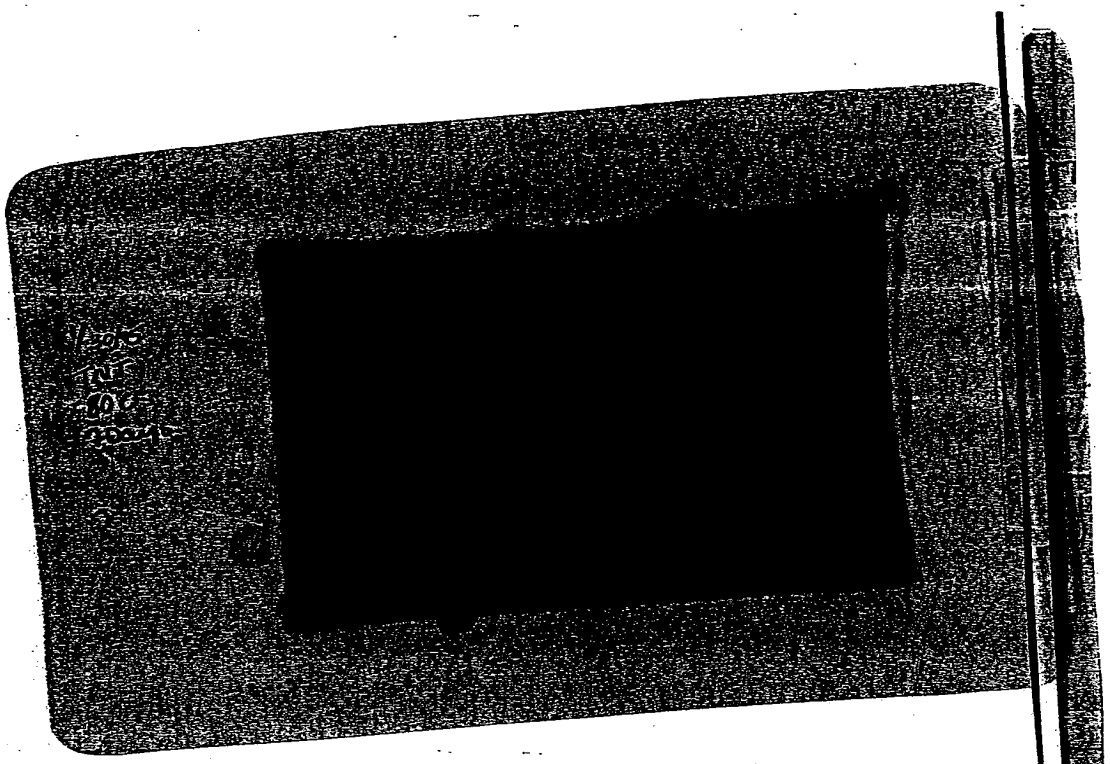
+PQE60 4-2
+PQE60

HTPB411515 + PA2 1

HTPBY11 + HTPAN08

Pg 42

1/23/95



+PAE60 4-2

HTPBY11S15 + PAZ 1

Make Glycerol stocks of all

800ul Bacterial Stock + 800ul 30% Glycerol

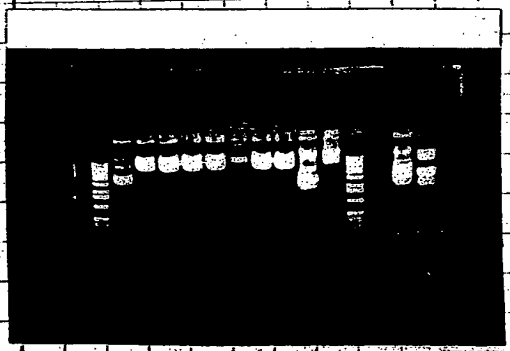
150

HIPAB08 7HRBY

1/23/95

Spin Culture 4.5 K 20 min
Pour off Supernatant
Resuspend pellet in 10 ml P1 + RNase
Let sit RT 5 min
Add 10 ml P2 - mix by scorling
~~Add~~ locked for cell Lysis √
Add 10 ml P3 -
Incubate on ice 30 min
Spin 8K 30 min
Prepare Column
apply 10 ml QBT
allow to flow through
Apply Supernatant to Column through
~~to~~ KDm cups
Allow to flow through
Wash Column 30 ml Wash Buffer
Elute DNA 15 ml QF
Add Isopropanol 0.7 vol (10.5 ml)
Mix well
Spin 8K 30 min
Pour off Supernatant
Wash Pellet 10 ml 70% Etanol (-20°C)
Spin 8K 15 min
Pour off Supernatant
Allow pellet to dry at RT for 10 min
Resuspend pelleted 400 ul TE pH 7.6

Run 1ul on gel with 1kb ladder
PAZ + DDEco



looks good

HTPAN08 / HTPB44

1/23/9

Read OD260/280.
Dilute 1:200 in H_2O

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm		400ul
1 [illegible]	0.0466	0.0293	0.0014	1.6230	0.6162	0.47ug/ul	188ug
2 HTPAN51 PAZ 1	0.1271	0.0721	-0.0021	1.7411	0.5743	1.3ug/ul	520ug
3 HTPAN51 PAZ 3	0.1186	0.0663	-0.0002	1.7859	0.5599	1.2ug/ul	480ug
4 HTPAN51 PAZ 4	0.0983	0.0547	-0.0023	1.7655	0.5664	1.0ug/ul	400ug
5 HTPAN185 PAZ 8	0.0919	0.0533	-0.0003	1.7201	0.5814	0.92ug/ul	368ug
6 " 3	0.0033	0.0001	-0.0053	1.5858	0.6306	X	
7 " 4	0.1070	0.0630	-0.0008	1.6905	0.5915	1.1ug/ul	440ug
8 5	0.1304	0.0771	0.0035	1.7244	0.5799	1.3ug/ul	520ug
9 HTPAN185 PQE4-1	0.0967	0.0605	0.0045	1.6471	0.6071	0.97ug/ul	358ug
10 HTPAN 185 PQE 4-1	0.0481	0.0287	0.0002	1.6798	0.5953	0.48ug/ul	192ug
11 HTPB44 PAZ 1	0.0502	0.0281	-0.0017	1.7434	0.5736	0.50ug/ul	200ug

Dilute DNA to ~~[illegible]~~ 250ng/ul.
Submitt for sequencing
for HTPAN08 - FR16 & RP06
for HMEAA85 RP01, RP02, RP04, Rev & For
HTPB44

Inoculate 300 ml LB+Amp +Kan
with 5ml HTPAN08 S04 185bp+PQE60
O/N culture. (4-1)
Incubate at 37°C w/aeration
until OD_{600} ≈ 0.4-0.6
Add IPTG to 2mM (6ml of 100mM IPTG)
Incubate at 37°C w/aeration
5 hours.
Spin culture 8K 20 min
Resuspend in 30 ml 6M GnHCl pH8
Store 4°C till tomorrow

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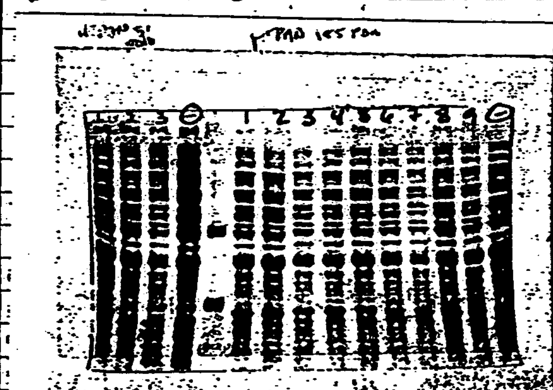
[signature]

HTPAN08 / HTPBY11

1/24/95

1/2 Inoculate 200 ul LB + Amp Kan
with ⊕ clones of #
HTPAN08 516p + PQE10 - 3
HTPAN08 185bp + PQ10 - 15 } 50ul
HTPAN08 516p + PQE60 - 17
Incubate w/ aeration 1 hour - 37°C
Add 100mM IPTG to 2mM → 25ul
Incubate 37°C w/ aeration 5 hrs.
HTPAN08 516bp + PQE60 — None Grew

Spin Cultures
Add 20ul H_2O to Resuspend pellet
Add 20ul 2X Dissociation Buffer
Heat 100°C 5min
Run on 10% Acrylamide gel with
Low Mol. Weight Marker.
150V 1 1/2 hrs.
Stain 30 min
DeStain 30 min



Not enough gels to run remaining samples, but does not look like anything induced.

Looks like from sequence, there is a problem with 3' Xba end —
Remake Primer

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